Identification of genetic and non-genetic factors contributing to female reproductive ageing

Submitted by

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

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Abstract

The aim of my work was to identify additional genetic and non-genetic factors influencing female reproductive ageing in humans. Although approximately 50% of population variation in age at menopause is due to genetics, less than 3% of variation had been accounted for by common genetic variants. Of non-genetic risk factors, only smoking had consistently been found to have a strong effect on age of menopause. In the wider context of female reproduction, our understanding of the role of genetics in determining sex hormone levels was limited. By combining the results of research in these different areas, I hoped to improve our knowledge of the biology of female reproductive ageing.

Chapter 1 is an introduction in which I discuss the biology of menopause, describe relationships with health and present current knowledge regarding non-genetic and genetic risk factors influencing menopause age.

Chapter 2 is an analysis of the associations between non-genetic risk factors occurring in early life with early menopause. We identified an association between multiple births and early menopause, connecting events pre-birth, when the oocyte pool is formed, with reproductive ageing in later life.

Chapter 3 is a genome-wide association study to identify genetic variants associated with levels of nine sex hormone related phenotypes. We highlighted loci of relevance to reproductive function, which suggested overlaps in the genetic basis of hormone regulation.

Chapter 4 is a genome-wide association study of menstrual cycle length. We showed that a common genetic variant related to follicle stimulating hormone levels and age at menopause is associated with several reproductive traits including length of menstrual cycle.

Chapter 5 is an investigation of the relationship between differences in length of normal *FMR1* triplet repeat alleles and timing of menopause. We found no association between the length of normal *FMR1* alleles and timing of menopause, contradicting the results of smaller studies and replicating a null result in another large study.

Chapter 6 is large genome-wide meta-analysis to identify common and low-frequency genetic variants associated with age at menopause. We identified 44

regions containing 54 independent common signals and two rare missense alleles of large effect.

Finally, in Chapter 7 I evaluate how this work has benefitted our knowledge of female reproductive ageing and describe directions for future research.

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I would also like to thank the *ReproGen* consortium for the opportunity to be involved as an analyst and for the opportunities they have given me to present the consortium's work at meetings. Thank you also to the other analysts I have worked with on their projects, particularly Dr Felix Day for his help.

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Author's Declaration

I was involved in the study design, analysis and manuscript preparation for all of the studies that are included as chapters in this thesis. Some of the studies include analyses performed by other authors, however in each case I had a major role and was the first or joint first author on the paper. My specific contributions for each chapter are listed below:

Chapter 1: Introduction

I conducted a literature review and wrote the content.

Chapter 2: The influence of early life events on premature reproductive ageing: analysis of cross-sectional survey data from the UK Biobank

I conducted the statistical analyses and prepared the manuscript.

Chapter 3: Genome-wide association study with 1000 genomes imputation identifies signals for nine sex-hormone-related phenotypes

I analysed the genome-wide association study results and prepared the manuscript.

Chapter 4: Genetic evidence that lower circulating FSH levels lengthen menstrual cycle, increase age at menopause, and impact female reproductive health

I defined the female reproductive variables, analysed the genome-wide association study results, carried out the association testing for the other reproductive phenotypes and prepared the manuscript.

Chapter 5: No evidence of an association between normal length FMR1 alleles and age at menopause in the general population

I conducted the statistical analyses and prepared the manuscript.

Chapter 6: Large-scale genomic analyses link reproductive ageing to hypothalamic signaling, breast cancer susceptibility and BRCA1-mediated DNA repair

I was a joint lead analyst on the project. I carried out quality control of the 24 new studies for the HapMap2 analysis and 22 studies for the Exome Chip analysis, ran the meta-analyses in parallel with another analyst, and was involved in checking and analysing the results. I carried out the molecular modelling and functional lookups described in Supplementary Tables S26 and S27. I contributed to writing the introduction and methods, and producing Figure 2, Table 2 and the supplementary materials.

Chapter 7: Discussion

I wrote the content.

Appendices

I carried out literature searches and updated and expanded existing tables provided by Dr Anna Murray.

Chapter 1: Introduction

Introductory notes

In this Chapter I discuss the biology of menopause within the context of reproduction in females. I describe the importance of age at menopause in relation to human health. Finally, I present current knowledge regarding non-genetic and genetic risk factors that influence age at menopause.

Menopause marks the end of female human reproductive lifespan

The process of menopause occurs in human females and marks the end of reproductive lifespan. Natural menopause occurs when there are too few ovarian follicles to drive the menstrual cycle, therefore a woman's ovarian reserve will determine her age at menopause. Usually, natural menopause occurs at around 50 years with most women having menopause between 45 and 55 years.

Menopause can occur at any point after the onset of menarche with 1% of women having menopause below the age of 40 years, a medical condition known as premature ovarian insufficiency (POI). Unlike menarche, which can be identified as starting at a distinct age, menopause is a process occurring over a number of years. Therefore, one commonly used definition of menopause is the cessation of menstrual periods with the date of last period occurring more than one year previously[1].

Menopause is a process that is almost unique to humans, with only a few other species thought to have post-reproductive lifespans, including gall-forming social aphids, killer whales and short-finned pilot whales[2,3]. Classical evolutionary theory suggests that a decline in reproductive ability before the end of life would be selected against and so there must be evolutionary advantages to menopause[3]. Gains in fitness for post-reproductive women have been suggested to result from reducing risk of mortality from pregnancy in later life, investing in their children (the 'mother hypothesis'), investing in their children's

children (the ‘grandmother hypothesis’) and reducing reproductive overlap between generations (‘reproductive conflict’)[3,4].

Ovarian reserve is determined before birth

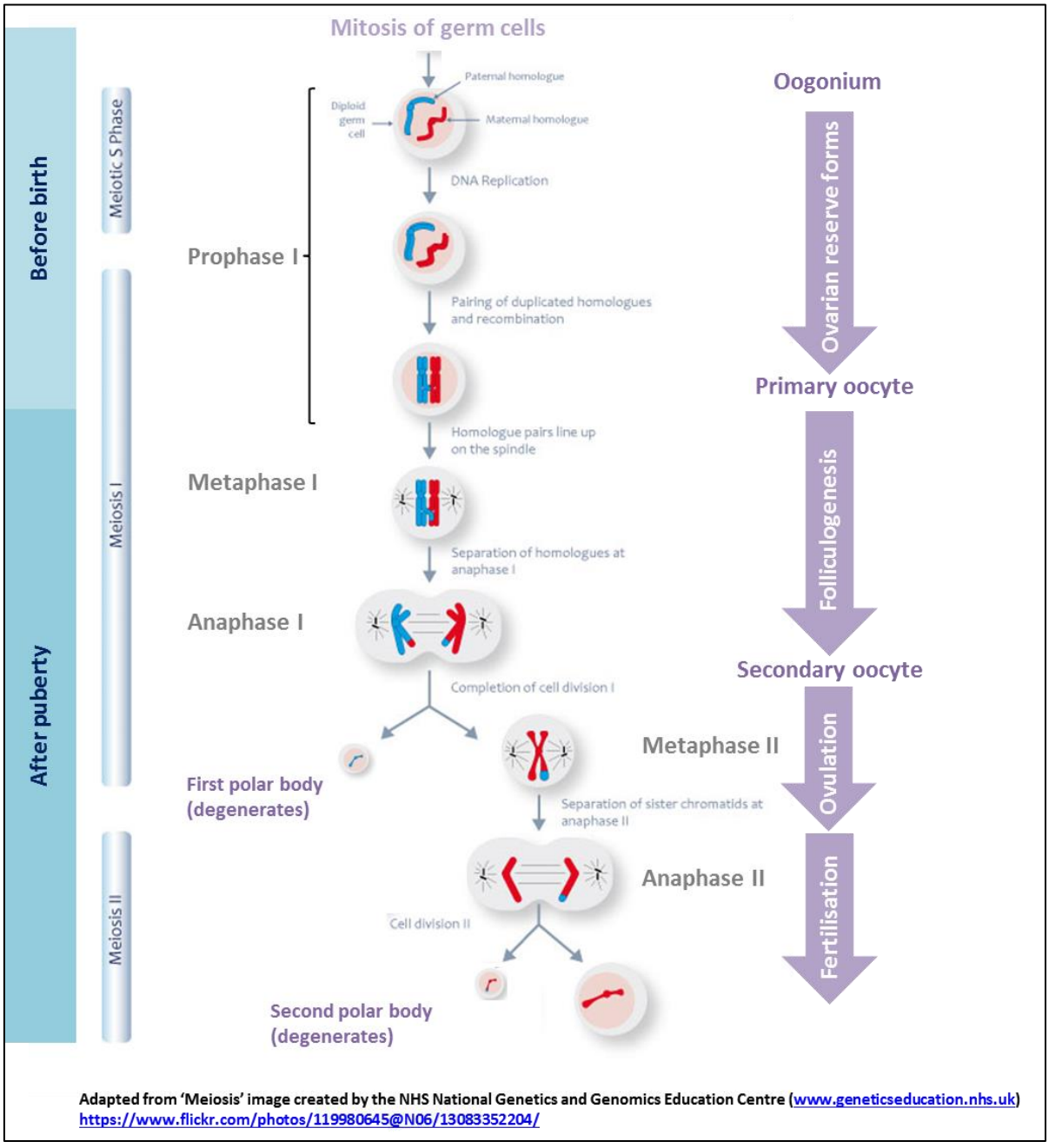
The number of ovarian follicles is determined before birth, with 6-7 million primordial follicles produced by around 4 month’s gestation, declining to only around 1,000 follicles by menopause[5] (Figure 1).

Figure 1. Number of ovarian follicles declines during a woman’s lifetime.

Oogenesis occurs before birth

Ovarian follicles are produced as a result of mitosis of germ cells and their differentiation to oogonia, which then enter meiosis to form primary oocytes in a process known as oogenesis (Figure 2). Oocytes enter meiosis over a period of several days according to position in the ovary[6]. Primary oocytes remain in prophase of meiosis I until puberty when cohorts of follicles start to develop, and are found in primordial follicles surrounded by a flattened layer of granulosa cells. As a result, many primordial follicles remain in state of dormancy for most of a woman’s lifetime. Recently human stem cells that can generate oocyte-like cells have been identified, suggesting that these could help maintain ovarian reserve, though the existence of these cells is controversial[6-8,9-12].

Figure 2. The process of oogenesis creates oocytes by meiosis.



Most oocytes are lost through atresia

Before birth a large number of follicles are lost by atresia prompted by apoptosis of the primary oocyte through mechanisms specific to the ovary in addition to conventional apoptotic pathways (Figure 1)[6,13-16]. Autophagy and necroptosis may also contribute to atresia[6]. The suggested reasons for this decrease include failure to complete mitosis or meiosis, unrepairable DNA damage, a lack of pre-granulosa cells and breakdown of oocytes while being restructured into

ovarian follicles[6]. This results in a rapid decline in the number of follicles (Figure 2), to approximately 300,000–400,000 by puberty[5].

A smaller number of oocytes are lost through ovulation

After puberty, the hormonal changes associated with menstruation drive the development of cohorts of follicles, and the concurrent production of secondary oocytes, as a result of completing meiosis I and starting meiosis II. Folliculogenesis occurs in waves, with the whole process taking about 120 days, and so the ovary contains follicles at all stages of development. Mature follicles contain a secondary oocyte surrounded by granulosa and thecal cells, and from these a dominant follicle is selected for ovulation. Unselected follicles undergo atresia driven by apoptosis of the granulosa cells[13]. Only oocytes that are fertilised will complete meiosis II and the remainder degenerate. Overall, the decrease in the number of oocytes over a woman's lifetime is mainly due to atresia before puberty, with only around 400 lost through ovulation.

Oocyte quality declines with age

A woman's ability to maintain the quality of oocytes throughout life will impact fertility and reproductive lifespan. Oocyte quality decreases with age as demonstrated by increased numbers of miscarriages in older mothers. The percentage of pregnancies that are trisomic increases from 2–3% for women in their 20s to over 30% for women in their 40s, with most aneuploidies originating from the mother[17-19].

It has been suggested that the oocytes created first are the first to be ovulated, and that they have more recombination events and a lower risk of non-disjunction – 'the production line hypothesis'[20]. However, there is no difference in recombination rates in oocytes from older women compared with those from younger women[21] or in the number of recombination events in oocytes entering meiosis early in foetal life compared with those created later[17]. Other explanations for aneuploidy relate to the extended period of time for which oocytes are arrested in prophase I. These include loss of cohesion between sister chromatids, age-dependent decay of components of the cell machinery required for meiosis and influence of environmental exposures[17,18].

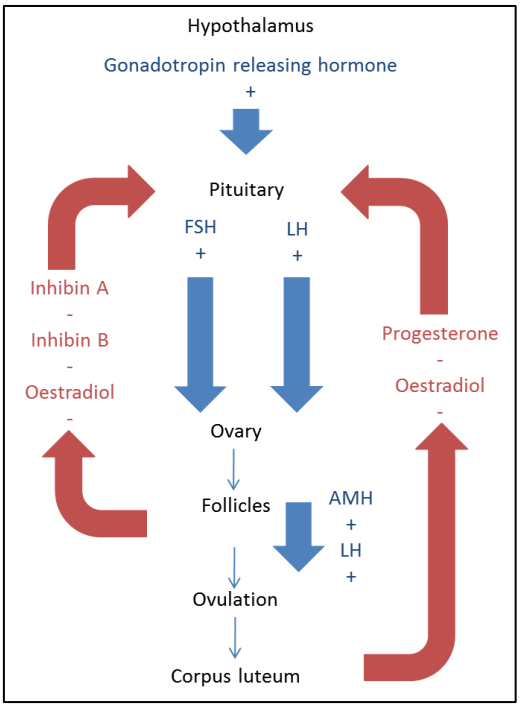
Menopause has effects on hormone levels

Towards menopause the menstrual cycle becomes dysregulated and ultimately ceases as a consequence of insufficient oocytes to maintain normal hormone levels.

Hormonal control of the menstrual cycle before menopause

The menstrual cycle is controlled by the hypothalamus via the pituitary gland, via pulsatile secretions of gonadotropin releasing hormone (GnRH) that cause the pituitary to secrete FSH and LH to control the ovaries[1]. Control of the cycle is maintained by feedback loops between hormones. FSH drives follicle development, causing release of inhibin A, inhibin B and oestradiol which then have a negative feedback effect on FSH levels[22] (Figure 3) . LH rises during follicle development and a surge in this hormone is required for ovulation[22]. Anti-Müllerian hormone (AMH) is thought to play a role in the selection of the dominant follicle and regulation of sensitivity of the developing oocytes to FSH[23]. After ovulation, the corpus luteum secretes progesterone, required for maintenance of the lining of the uterus, and to a lesser extent oestradiol. The breakdown of the corpus luteum and the resulting fall in progesterone causes a new cycle to begin.

Figure 3. The menstrual cycle is controlled by hormonal feedback.



Hormone levels change around menopause

In the run up to menopause, declining numbers of ovarian follicles (to around 1000) results in a decrease in AMH and inhibin B. Loss of this negative feedback on FSH and LH, and loss of ovarian responsiveness to these gonadotropes, results in rising GnRH, FSH and LH levels[24]. Increased FSH can lead to an 'overshoot' in oestrogen production, prompting the LH surge, accelerating ovulation and shortening the follicular phase of the menstrual cycle[1,24]. Towards menopause, oestrogen levels are erratic, progesterone levels decline[25], menstrual cycles become irregular and anovulatory cycles become more frequent[26].

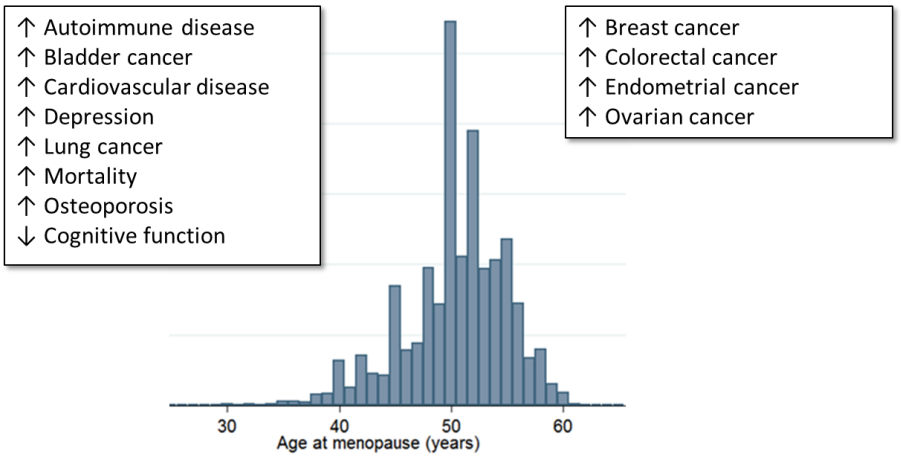
Hormone levels after the menopause

After menopause, ovulation and menstruation no longer take place. As a result of very low numbers of remaining ovarian follicles, the ovaries stop producing oestradiol and progesterone. AMH and inhibin B levels are very low and FSH levels stabilise at elevated levels[1]. However, the ovary continues to produce androstenedione and testosterone at pre-menopausal levels[26]. Although oestradiol is not produced by the ovaries, in postmenopausal women the main oestrogen produced is oestrone, synthesized from androstenedione in the liver, kidney, brain, adrenal and peripheral adipose tissue[26].

Effects of age at menopause on health

Age at menopause is associated with wide-ranging effects on female health in addition to impacting fertility (Figure 4) (Table1, Appendix 1). Many of these associations have been seen in different countries and ethnicities. The health effects of age at menopause are suggested to be a consequence of length of time exposed to female sex hormones, with longer exposure being protective against some diseases, for example heart disease, but detrimental for others, for example, breast cancer. The association of a wide range of ageing-related adverse health outcomes and increased mortality with early menopause suggests that menopause may be a more general ageing process[27].

Figure 4. Early and late menopause are associated with a range of health outcomes.



Source of menopause age distribution: UK Biobank

Effects of early menopause

Mortality

Earlier menopause is associated with increased risk of death from all causes[28-38], with one study estimating a 1.6% increased risk of mortality per 3 year decrease in menopause age [33]. More specifically, earlier menopause is associated with increased risk of mortality from cancer[32,36,38,39], cardiovascular disease[32,38,40,41], external causes[34], genitourinary disease[34] and respiratory disease[34].

Bone density

Decreased bone mineral density[42], increased osteoporosis[29,43] and increased risk of bone fractures[28,29,44,45] are all associated with earlier menopause. One study estimated the odds of osteoporosis to be increased by 3% per year decrease in menopause age[43]. Another estimated the odds of bone fracture to be increased by 50% in women with menopause under 45 years compared with over 45 years[44]).

Cardiovascular disease

Women with earlier menopause are at increased risk of coronary heart disease[46-49] and stroke[46,50], with women with menopause under 45 years

estimated as being at about twice the risk compared with those with menopause at over 45 years[46]. Earlier menopause is also associated with increased cholesterol[51], atherosclerosis[52] and heart failure[53,54]. However, both early and later menopause are associated with increased risk of venous thromboembolism[55,56].

Diabetes

Studies have shown higher odds/risk of type II diabetes in post-menopausal women (40% higher odds compared with pre-menopausal)[57] and women with earlier menopause (30% higher risk for menopause aged under 40 years compared with 45–49 years)[58].

Auto-immune diseases

POI is associated with a 50% increased risk of autoimmune diseases [36], while more specifically, earlier menopause is associated with lupus, rheumatoid arthritis and sarcoidosis[36,59-61].

Cancers

Several cancers are associated with early menopause with an increased risk of bladder cancer[62,63], lung cancer[64,65], upper gastrointestinal tract cancer[66] and pancreatic cancer[67] by about 30–60%. Other outcomes associated with earlier menopause include depression[68,69], gout[70], lower back pain[71], glaucoma[72], larger aortic aneurisms[73] reduced cognitive function[74,75] and lower walking speed[76].

Effects of later menopause

In contrast, later menopause is associated with increased risk of the female-specific cancers breast[36,77-79], endometrial[80,81], ovarian[82] cancer and increased risk of mortality from ovarian or uterine cancer[32]. The odds of breast cancer are increased by nearly 3% per year increase in age at menopause [83], while risk of endometrial cancer is twice as high in women with menopause aged over 55 compared with 50 and under [80]. In addition, later menopause has been found to be associated with increased risk of colorectal cancer by 50% in women with menopause at over 55 years compared with under 40 years[84].

Table 1. Health outcomes associated with age at menopause. (For full version of table see Appendix 1).

Outcome	Effect on outcome	Ref.
Aortic aneurism	Earlier menopause → bigger aortic aneurism	73
Autoimmune diseases	POI → increased risk of autoimmune disease	36
Autoimmune, rheumatoid arthritis	Early menopause - > milder rheumatoid arthritis	85
	Early menopause → increased rheumatoid arthritis	60
Autoimmune, sarcoidosis	Earlier menopause → increased risk of sarcoidosis	61
Autoimmune, systemic lupus erythematosus	Earlier menopause → increased risk systemic lupus erythematosus	59
Bone, bone mineral density	Earlier menopause → decreased bone density	42
Bone, fractures	Early menopause → increased fractures	28,29, 44,45
Bone, osteoporosis	Early menopause → increased osteoporosis	29,43
Cancer, bladder	Earlier menopause → increased risk of bladder cancer	62,63
Cancer, breast	Later menopause - > increased risk ductal breast cancer	77
	Later menopause - > increased risk lobular breast cancer	77
	Later menopause → increased breast cancer risk	78,83
	Later menopause → increased risk of ductal carcinoma in situ	79
	POI → reduced risk of breast cancer	36
Cancer, colorectal	Later menopause → increased colorectal cancer	84
Cancer, endometrial	Later menopause → increased risk of endometrial cancer	80,81
Cancer, lung cancer	Earlier menopause → increased risk lung cancer	64,65
Cancer, pancreatic	Early menopause → increased risk pancreatic cancer	67
Cancer, upper GI tract	Earlier menopause → increased risk of squamous cell carcinoma of the upper GI tract	66
	Post-menopausal → increased risk of oesophageal carcinoma	86
Cognitive function	POI → long-term reduced cognitive function	74
	Early menopause → increased odds of cognitive impairment	75
Cancer, ovarian	Later menopause → increased risk of ovarian cancer	82
CVD	Being menopausal → increased CVD	87
	Earlier menopause → increased risk of CVD in women with RA	88
CVD, atherosclerosis	Earlier menopause → increased atherosclerosis	52
CVD, cholesterol levels	Early menopause → increased odds hypercholesterolaemia	51
CVD, coronary heart disease	Early menopause → increased heart disease	46-48
	Early menopause → possibly increased heart disease	49
CVD, deep vein thrombosis	Early and late menopause → increased risk of DVT	55
CVD, heart failure	Early menopause → increased heart failure	53
CVD, hypertension	POI → reduced risk of hypertension	36
CVD, NT-proBNP levels	Early menopause → increased N-terminal pro brain natriuretic peptide (NT-proBNP) levels (marker for CVD and heart failure)	54
CVD, stroke	Early menopause → increased stroke	46
	POI → increased risk of cerebral infarction	50
CVD, venous thromboembolism	Earlier menopause → increased risk VTE	56
	Early and late menopause → increased risk of VTE	55
Depression	Early menopause → increased risk of depression	68
	POI → higher prevalence of depression in lifetime	69
Diabetes, dysglycaemia	Post-menopausal → higher odds dysglycaemia	57
Diabetes, pre-diabetes	Post-menopausal → higher odds pre-diabetes	57
Diabetes, type II	Earlier menopause → increased risk of Type II diabetes	58

Outcome	Effect on outcome	Ref.
	Post-menopausal → higher odds diabetes	57
Glaucoma	Earlier menopause → increased risk of glaucoma	72
Gout	Earlier menopause → increased risk of gout	70
Low back pain	Early menopause → increased odds of low back pain	71
Mortality, all causes	Early menopause → increased all cause mortality	28-35
	POI → increased mortality from all causes	36-38
Mortality, cancer	Early menopause → increased risk cancer mortality	39
	POI → increased risk mortality from cancer	36,38
Mortality, coronary heart disease	Earlier menopause → increased risk of death from heart disease	30,32, 34
Mortality, CVD	Earlier menopause → increased risk of death from CVD	32,40, 41
	POI → increased risk of death from CVD	38
Mortality, external causes	Early menopause → higher risk of death from external causes	34
Mortality, genitourinary disease	Early menopause → higher risk of death from genitourinary disease	34
Mortality, ovarian or uterine cancer	Later menopause → increased risk of death from ovarian or uterine cancer	32
Mortality, respiratory disease	Early menopause → higher risk of death from respiratory disease	34
Walking speed	Early menopause → slower walking in old age	76

Non-genetic causes of variation in age at menopause

A wide range of epidemiological risk factors are associated with age at menopause (Table 2, Appendix 2).

Smoking

Being a current smoker is associated with reduced age at menopause in 24 epidemiological studies in different ethnicities[89-112]. Smoking is estimated to reduce age at menopause by over a year[97]. In the largest such study of over 50,000 women, Morris et al found that current smokers were 30% more likely to be menopausal compared with women who never smoked[89].

Fewer studies have looked at the effect of amount smoked or time smoked on age at menopause and results have been less clear. However, a large study on over 33,000 women showed a reduction in age at menopause by 0.3 years for smoking for more than 28 years compared with smoking for less than 28 years[90]. Being a former smoker is associated with earlier menopause though to a lesser extent than being a current smoker[89,92,94,98,105,108,113].

The biological effects of cigarette smoking on age at menopause have been suggested to be due to loss of ovarian follicles due to the toxins in cigarette smoke[114]. Also smokers may have lower oestrogen due to anti-oestrogenic effects of cigarette smoke and more rapid oestrogen metabolism[114].

Socio-economic status

Outcomes indicating lower socio-economic status are generally associated with earlier menopause. The most frequently studied such outcome in relation to age at menopause is educational level and lower levels of education have been consistently found to be related to earlier menopause in 17 studies in different countries, even after adjusting for the effects of smoking[39,91-93,98,99,104,106,108,115-122]. Menopause is estimated as being up to 1 year earlier in women with basic education compared with a university education[92] and such women are 15% more likely to be menopausal[93,115].

More generally, lower socio-economic status is associated with earlier menopause as is lower occupational class and being unemployed. The effect of socio-economic status on age at menopause is likely to be contributed to by

numerous differences in lifestyle, however it persists event with adjustment for reproductive factors, smoking, childhood socio-economic position and BMI[123].

Diet

There is less evidence for effects of diet on age at menopause, with alcohol consumption being the best characterised association. Results of several large studies suggest that alcohol consumption is associated with later menopause, with an 10% lower risk of early menopause[89,117,118]. This may be because moderate alcohol consumption leads to higher oestrogen levels[89].

Various associations with diet have been suggested. Later menopause is associated with higher intake of calories, cereals, fat, fruit, meat, protein, low fat dairy products and taking vitamin/mineral supplements[90,102,117,124], while earlier menopause is associated with higher cereal, vegetable and fibre intake and being vegetarian[89,90,102]. Carbohydrates have been associated with both earlier and later menopause[90,102].

Exercise

Studies on the effects of exercise have shown contradictory results but the larger studies have tended to show an association between higher exercise and later menopause[89,90] and earlier menopause in women who do not exercise[92,117,125]. The effect of exercise on age at menopause is small, with the largest study showing only a 4% reduction in risk of early menopause with regular strenuous exercise compared with no exercise [89].

Environmental toxins

Exposure to various environmental toxins have been found to be associated with earlier menopause. Exposure to diethylstilbesterol is associated with an over 40% increased risk of early menopause[115,126] while exposure to radiation reduces age at menopause by 0.3 years[127]. Exposure to other environmental toxins have shown smaller effects and have only been evaluated in smaller studies. Such exposures may cause endocrine disruption or have toxic effects on ovarian follicles.

BMI

Body mass index (BMI) is the best characterised anthropometric measure in relation to age at menopause. Ten studies have shown that lower BMI is associated with earlier menopause [89-91,93,94,96,113,128-130], including the largest epidemiological study to date on over 95,000 women in which women with a BMI of over 30 were 8% less likely to be menopausal compared with a BMI under 20[113]. In contrast one smaller study on approximately 5,000 women showed that earlier menopause is associated with higher BMI[100]. In addition, women with weight gain during lifetime are 7% less likely to be menopausal[89] and have a 0.4 year increase in menopause age [90].

Early-life events

There is evidence that events in early life are associated with age at menopause, though studies have been smaller. Earlier menopause is associated with food deprivation in childhood (by 1.8 years)[131] and during pregnancy (30% more likely to be menopausal)[132]. Lower socio-economic status in childhood is associated with earlier menopause by 0.6 years[123]. In contrast, both being breastfed[133,134] and heavier weight in infancy[133-135] are associated with later menopause. Both very low and very high birthweights are associated with increased chance of early menopause [136].

Associations between early-life events and age at menopause are discussed further in Chapter 2, where I present analyses in over 185,000 women from the UK Biobank.

Reproductive factors

Of reproductive factors, most evidence is for an association between increased numbers of births and later menopause. Women with three or four children are 12–27% less likely to be menopausal than women with no children[89,90,113]. Earlier menarche is associated with earlier menopause in a number of studies [90,91,93,98,113,128,137] with a 1-2% decrease in risk of early menopause[113] or a 0.1 year increase in age at menopause[91] with each 1-2 year increase in age at menarche. However, analysis of over 115,000 women in UK Biobank showed that earlier and later menarche are both associated with earlier menopause[138].

There is conflicting evidence about the effect of contraception, though on the whole oral contraceptive use is associated with later menopause.

Trend over time

There is a secular trend in age at menopause with age at menopause increasing over time[96,99,100,105,129,137,139]. Age at menopause is estimated to increase by over a month per year of birth[137,139].

Ethnicity

Age at menopause varies according to ethnicity and geographic region. A recent meta-analysis estimated a mean age at menopause of 47.2 years in Latin-America, 47.4 years in the Middle East, 48.4 years in Africa, 48.8 years in Asia, 48.8 years in the USA, 50.5 years in Europe and 51.3 years in Australia [140]. These differences are likely to be due to a combination of ethnicity and differences in socio-economic status and lifestyle factors. In a US study, Japanese Americans were 7% less likely to be menopausal compared with white Americans, while Latin Americans were 10% more likely to be menopausal[113].

Table 2. Epidemiological risk factors associated with age at menopause. (For full version of table see Appendix 2).

Variable	Effect	Reference
Alcohol	Alcohol → later menopause	99,106,117
	Higher alcohol consumption → later menopause	89,111
	Increase in alcohol consumption → later menopause	118
BMI	High BMI → earlier menopause	100
	Lower BMI → earlier menopause	89-91,93,94,96,113,128-130
BMI, change	Episodic weight loss >5kg → later menopause	109
	Greatest increase in BMI from age 25 to menopause → later menopause	109
Childhood, birth weight	Extreme birthweight → earlier menopause	136
	Heavier birthweight of twin → earlier menopause	141
Childhood, breastfed	Breastfeeding → later menopause	133
	Breastfeeding for longer as child → later menopause	134
Childhood, cognitive ability	Lower cognitive score as child → earlier menopause	142
Childhood, foetal growth	Faster foetal growth → earlier menopause	136
Childhood, food deprivation	Exposure to famine at 2-6 years → earlier menopause	131
Childhood, gestational food deprivation	Gestational exposure to famine → earlier menopause	132
Childhood, maternal smoking	Exposure to maternal smoke in pregnancy → earlier menopause in never smokers	143
Childhood, parental divorce	Parental divorce as young child → earlier menopause	144
	Parental divorce during childhood → earlier menopause	133
Childhood, SES	Crowding at 2 years → earlier menopause	134
	Lower SES as child → earlier menopause	144
		123
	No bathroom in house as child → earlier menopause	123
Childhood, siblings	Fewer siblings → later menopause	105
Childhood, weight	Heavier at 2 years → later menopause	133
	Higher weight at 2 years old → later menopause	134
	Lower weight at age 1 year → earlier menopause	135
Cognitive ability	Increasing cognitive ability → later menopause	133
Diet, calories per day	Higher calories → later menopause	90
Diet, carbohydrates	Higher carbohydrate intake → earlier menopause	102
	Higher carbohydrate intake → later menopause	90
Diet, cereals	Higher cereal product intake → earlier menopause	102
Diet, fat	Higher fat intake → later menopause	102
	Low fat dairy products → later menopause (in women premenopausal at <51 years)	124
Diet, fibre	Higher fibre intake → earlier menopause	102
Diet, food deprivation	Exposure to famine → earlier menopause	131
Diet, fruit	Higher fruit intake → later menopause	90
Diet, meat	Higher meat intake → later menopause	102
Diet, protein	Higher protein intake → later menopause	90
Diet, vegetables	Higher vegetable intake → earlier menopause	102
	Vegetarian → earlier menopause	89
Diet, vitamins/minerals	Supplementation with vitamins/minerals → later menopause	117
Education	Less education → earlier menopause	39,91-93,98,99,104,106,108,115-122

Variable	Effect	Reference
Environmental exposure, combustion by-product	Higher 1,2,3,4,6,7,8-heptachlorodibenzofuran → earlier menopause	145
Environmental exposure, coolants	Polychlorinated biphenyl congeners (PCBs) → earlier menopause	145
Environmental exposure, lead	Exposure to lead → earlier menopause	146
Environmental exposure, pesticide	Higher organophosphate pesticide → earlier menopause	145
Environmental exposure, plasticiser	Higher mono-(-2-ethyl-5-hydroxylhexy)/ mono-(-2-ethyl-5-oxohexyl) pthalate→ earlier menopause	145
Environmental exposure, pre-natal oestrogen	Diethylstilbestrol exposure in utero → earlier menopause	115
	Pre-natal exposure to diethylstilbesterol → earlier menopause	126
Environmental exposure, radiation	Radiation exposure → earlier menopause	127
Exercise	Higher exercise → earlier menopause	118
	Higher exercise → later menopause	89,90
	Low exercise → earlier menopause	100
	Medium exercise → earlier menopause	107
	No exercise → earlier menopause	92,117,125
Geographical region, Europe	Czech Republic or Russia (compared with Poland) → earlier menopause	117
Geographical region, USA	Southern USA → earlier menopause	92
Health, blood pressure	Increasing blood pressure premenopause → earlier menopause	147
Health, cholesterol change premenopause	Decreasing cholesterol level premenopause → later menopause	147
	Increasing cholesterol premenopause → earlier menopause	147
Health, cholesterol premenopause	Higher premenopausal cholesterol → earlier menopause	147
Health, heart disease	Heart disease → earlier menopause	92,116
Health, heart disease risk score	Increased Framingham risk score → earlier menopause	147
Health, premenopausal T2 diabetes	T2 diabetes → earlier menopause	109
Health, self-reported	Poorer health → earlier menopause	98,118
Height, adult	Short height → earlier menopause	89,123
Marital status	Married → later menopause	95,121,148
	Separated/widowed/divorced → earlier menopause	116
Maternal age at menopause	Increasing age of mother at menopause → later menopause	133
Race/ethnicity	Japanese → later menopause	113,116
	Latina → earlier menopause	113
	Latin-American immigrant → earlier menopause	122
	Non white ethnicity → earlier menopause	39
Refugee	Refugee → earlier menopause	149
Religion	Jewish → later menopause	150
Reproductive, age at birth of first daughter	Later age at birth when had daughter → later menopause	151
Reproductive, age at first birth	Younger age at first birth → earlier menopause	39
	Younger age at first live birth → later menopause	90
Reproductive, age at first pregnancy	Younger at first pregnancy → earlier menopause	102,121
Reproductive, age at last birth	Younger age at last live birth → earlier menopause	90

Variable	Effect	Reference
Reproductive, age at last pregnancy	Younger age at last pregnancy → earlier menopause	152
Reproductive, birth control	Intrauterine device → later menopause	90
	Long-term oral contraceptive use → earlier menopause	96
	Oral contraception → earlier menopause	152
	Oral contraceptives → earlier menopause	153
	Oral contraceptives → later menopause	90,94,98,105,116-118,121
	Tubal sterilisation → later menopause	90
	Tubal sterilization → earlier menopause	153
Reproductive, endometriosis	Endometriosis → earlier menopause	128,153
Reproductive, fertility	Longer interval between marriage and first child → earlier menopause	105
Reproductive, HRT	HRT → later menopause	153
Reproductive, infertility	Infertility → earlier menopause	128
Reproductive, menarche	Earlier and later menarche → earlier menopause	99
	Earlier menarche → earlier menopause	90,91,93,98,113,128,137
Reproductive, menarche to first birth	Increased time from menarche to first birth → earlier menopause	90
Reproductive, menstrual cycle	Longer time from menarche to regular menses → later menopause	102
	Menstrual cycle irregularity → later menopause	91,129
	Menstrual cycle irregularity before age 25 → later menopause	120
	Shorter menstrual cycle → earlier menopause	98,154
Reproductive, number of births	More births → earlier menopause	104
	More births → later menopause	89-93,96,98,99,102,103,105,113,120,122,129,133,149,150,154
	No births → earlier menopause	104,116,152
	No births/one birth → later menopause	100
Reproductive, number of pregnancies	More pregnancies → later menopause	128,154
	Never pregnant → earlier menopause	39,107,152,154
Reproductive, time spent breastfeeding	Breastfed children → later menopause	102
Reproductive, unilateral oophorectomy	Unilateral oophorectomy → earlier menopause	129,155
Season of birth	Spring → earlier menopause; Autumn → later menopause	156
SES	Lower SES → earlier menopause	96,99,105,123
SES, employment	Not employed → earlier menopause	116,118
SES, housing tenure	Non-home owner → earlier menopause	144
SES, income	Financial hardship → later menopause	144
	Higher income → later menopause	99
	Lower income → earlier menopause	92,93,120
SES, lifetime	More adverse indicators across life course → earlier menopause	123
	More lifetime adversity → earlier menopause	104
SES, occupation	Higher occupational class → later menopause	121
	Lower occupational class → earlier menopause	99,104,119
Smoking	Ever smoker → earlier menopause	128
	Smoking → earlier menopause	118,153

Variable	Effect	Reference
Smoking, current	Current smoking → earlier menopause	89-112
	Higher amount smoked → earlier menopause	94,105,150,157
	Higher amount smoked per day → earlier menopause	108,116,117
	Increased amount smoked during life → earlier menopause	108
	Longer smoking → earlier menopause	90
	Smoking → earlier menopause	158
	Smoking → earlier menopause	39
	Smoking 10+ cigarettes per day → earlier menopause	159
	Smoking 30+ pack-years → earlier menopause	160
	Smoking around time of menopause → earlier menopause	115
Smoking, ever	Ever smoker → earlier menopause	105,129
	Ever smoking → earlier menopause	160
Smoking, passive	Passive smoking → earlier menopause	101
Smoking, previous	Ex-smoker → earlier menopause	113
	Higher amount smoked previously → earlier menopause	105,113
	Previous smoker → earlier menopause	92,94,98
	Previous smoking → later menopause	89
	Stopped smoking >10 years before menopause → later menopause compared with current smokers	108
Social participation	Higher social participation → later menopause	108
Weight	Higher adult weight → later menopause	89
	Higher weight → later menopause	118
	Higher weight age 20 → later menopause	90
Weight, change	Higher weight gain from 20-40 years → later menopause	89
	Increased weight premenopause → later menopause; decreased weight premenopause → earlier menopause	147
	Weight gain → later menopause	90
Year of birth	Earlier year of birth → earlier menopause	96,99,100,105,129,137,139

Genetic causes of variation in age at menopause

Age at menopause is strongly associated with mother's age at menopause[133], and heritability studies have estimated that over 50% of population variation in age at menopause is due to genetic variation[161-164]. Causes of genetic variation include rare, single gene mutations that change menopause age by many years and lead to POI, and common genetic variants that occur at allele frequencies of over 5% in the population and change at menopause by months, with low frequency variants with larger effect sizes filling the gap between (Table 3, Appendix 3).

Approximately 80 genes are thought to cause POI in humans. These genes have been identified by studying families with conditions such as POI, primary amenorrhoea, gonadal dysgenesis and hypergonadotropic hypogonadism sometimes occurring in conjunction with other medical conditions, for example, Perault syndrome, Nijmegen breakage syndrome and white matter abnormalities in the brain. Such genes include those involved with diverse processes such as control of the cell cycle, DNA damage response, DNA repair, embryonic development, metabolism, gene expression, hormone signalling, immune function, meiosis, protein synthesis and gonad development.

In recent years, common genetic variants have been identified by genome wide association studies (GWAS) that most recently have led to the identification of 44 genetic loci associated with approximately 6% of common variation in age at menopause[165-170], including loci involved in immune function, DNA repair, POI and disorders of puberty timing (described in Chapter 6)[170]. GWAS have largely been in white European women, though some signals have been replicated in other ethnicities. While GWAS studies have identified genetic loci, identifying causal variants and genes is more challenging. The most likely causal genes are suggested on the basis of their relationship with the strongest signal in a region, for example, physical proximity, being in linkage disequilibrium, demonstrating changes in expression levels and biological plausibility. Therefore, many of the genes identified by GWAS remain to be confirmed as functionally important by *in vitro* studies.

Genes causing POI and normal variation in age at menopause

Homologous recombination

A number of genes have been identified both from studies of POI and GWAS that are involved in homologous recombination; *MCM8, MSH5* and *DMC1*. A common variant in *MCM8*, a DNA helicase involved in DNA repair, is associated with a decrease in age at menopause by up to one year[165-170], while a homozygous mutation in this gene resulted in deficiency of double strand break repair in three sisters with amenorrhea and hypergonadotropic hypogonadism[171]. Common variants near *MSH5*, which is involved in meiotic recombination, have been found to reduce age at menopause by 0.16 years [168,170,172], and a heterozygous missense mutation causing POI has also been found in this gene[173].

Auto-immunity

The involvement of auto-immunity in age at menopause has been suggested by associations of a loci near the *HLA* region with normal variation in age at menopause[168,170,172]. Certain *HLA* types have been found to be associated with POI[174,175].

Hormone levels

Variants in *FSHB* have been identified associated with a reduction in age at menopause of 0.1–0.25 years[168,170,176]. Frameshift deletions in this gene lead to FSH deficiency and amenorrhea[177-179].

Other genes

A common variant near *POLG*, required for replication of mitochondrial DNA, reduces age at menopause by 0.18 years[168,170,176] while dominant mutations are associated with progressive external ophthalmoplegia and POI. Also highlighted by GWAS and POI studies is *EIF2B4*, a subunit of eukaryotic initiation factor 2B (*EIF2B*), which is involved in protein synthesis.

Other genetic causes of POI

Fragile X premutation

The most common cause of POI are Fragile X premutation length repeats in the *FMR1* gene, which have been estimated as occurring in 2% of POI cases[180]. The 5` untranslated region of the *FMR1* gene contains a CGG repeat that varies in length causing Fragile X syndrome at full length mutations of over 200 copies, with repeat lengths of 55–200 copies considered to be premutation lengths for Fragile X.

Women with Fragile X premutation length repeats in *FMR1*[181,182] have an increased risk of POI and early menopause as well as altered menstrual cycles[183]. However, women with full length Fragile X mutations retain normal ovarian function. Studies have suggested that variation in normal length FMR1 repeats might be associated with differences in age at menopause[184-187], however these results have been debated[187]. In Chapter 5 I describe efforts to resolve this controversy.

Gonad formation and development

A number of mutations causing POI have been identified in genes that are involved in gonad formation and development. About 20 different mutations have been identified in *BMP15*, an oocyte specific growth factor that simulates folliculogenesis[188-191]. Many mutations affecting protein structure and function have been identified in *FOXL2*, the forkhead box protein L2, a transcriptional regulator required for differentiation of the ovary[192-198].

Multiple missense mutations have been identified in *GDF9*, growth differentiation factor 9, which is required for folliculogenesis and granulosa cell proliferation[190,199-203] and also *NOBOX*, a transcription factor which when absent causes sterility in mice[204,205]. While several putative mutations have been identified in *DAZL*, an RNA-binding protein required for gametogenesis in both males and females[206].

Hormone function

Many mutations causing POI are in genes involved with hormone function. Missense mutations in the *AMH* gene have been identified that result in mutant

AMH with loss of AMH-receptor activation[207]. Mutations in *INHA*, inhibin alpha-chain, have also been found in POI[208-213].

As well as mutations in *FSHB* (discussed above), several mutations with functional effects have been identified in *FSHR*, the follicle stimulating hormone receptor[214-220]. In-frame, frameshift and missense mutations have been identified in *NR5A1*, a transcriptional regulator of genes involved in hormone regulation by the hypothalamic-pituitary-gonadal and adrenal axis[221-230].

DNA repair

Several genes causing POI are involved in DNA repair. *MCM9,* a DNA helicase and component of the minichromosome maintenance complex with *MCM8*, has been identified as causing primary amenorrhea in two families[231]., A higher frequency of certain haplotypes of genetic variants in *FANCA* (the Fanconi anaemia complementation group A gene) are found in women with POI[232]. Mutations in *SETX*, a probable RNA/DNA helicase, have been found in women with oculomotor apraxia type 2 and POI[233,234].

Meiosis

A number of genes involved in meiosis have been identified as causing POI, in addition to *MSH5*. Compound heterozygous mutations in *HFM1*, which is required for homologous recombination in mice, have been found in women with POI[235]. A homozygous deletion in *PSMC3IP* was identified in females with gonadal dysgenesis[236]. Truncation of the protein product of *STAG3* was identified in a family with POI and mice homozygous for this mutation were sterile[237]. Finally, two studies have identified mutations in *SYCE1* in several women with POI[238,239].

Other genes

Other functions of genes linked to POI include regulation of the cell cycle (*CDKN1B*[240]), cell survival (*HAX1*[241]), cell growth and proliferation (*RET*[242], *TGFBR3*[243]), stress signalling (*CLPP*[244]), fatty acid metabolism (*ACSL6*[245]), immune response (*XPNPEP2*[246]), protein synthesis (*NANOS3*[247-249], *RPL10*[250], *LARS2*[251]), transcription (*AFF2*[252,253], *POU5F1*[254]) and mitochondrial respiration (*COX10*[255]).

Genetics of normal variation in age at menopause

Homologous recombination

Pathway analysis of the genetic loci identified by the most recent GWAS of age at menopause[170]highlighted the role of homologous recombination (see Chapter 6). Of the genes identified, 12 are involved in homologous replication – *BRCA1, BRE, DMC1, FAM175A, FANCI, FBXO18,HELQ, MCM8, MSH5, RAD51, RAD54L, UIMC1*. Several of these genes have been identified as causing POI (*MCM8, MSH5, DMC1*). Additionally, BRCA1/2 carriers have a reduced age at menopause[256]. Such genes may have an effect on oogenesis perhaps influencing the oocyte pool, since homologous recombination is a key step in meiosis.

DNA damage response

Other genes identified from GWAS[168,170]are in other parts of the DNA damage response pathway. These include genes involved in DNA repair (*APTX, EXO1, REV3l, PAPD7, APEX1, PARP2, MSH5, MSH6*), replication (*PRIM1, HELB, POLG*), transcription (*POLR2E, POLR2H*), chromatin remodelling (*TLK1, CHD7, INO80*), sensing DNA damage (*BRSK1*), cell signalling (*CHEK2*), cell cycle control (*MLF1IP, KNTC1, CDK2AP1, MYCBP*) and apoptosis (*DIDO1, PARL*).

Puberty timing

The overlap between disorders of puberty is seen not only with POI but also with age of menopause in the normal range. Several genes identified are known to cause monogenic forms of hypogonadotropic hypogonadism (*CHD7, FGFR1, SOX10, KISS1R (GPR54), TAC3*)[170].

Immune function

In addition to *HLA*, a wider role for immune function genes is suggested[168]. Pathway analysis identified the genes *IL11, NLRP11* and *PRRC2A (BAT2)* near signals for age at menopause in a GWAS in 2012[168], with *NLRP11* also identified as a potential candidate by the most recent GWAS[170]. The genetic signal near *NLRP11* and has been replicated in other ethnicities[167,169,257].

Telomere length

There is some evidence that telomeres may play a role in determining menopause age, with some studies showing longer telomeres associated with later menopause and others finding no association[27,258,259]. Telomeres are repetitive sequences at the ends of the chromosome that shorten during successive cycles of mitosis, which at a critically short length prompt cell cycle arrest and apoptosis. Telomere shortening has been implicated in other ageing processes and suggested to be associated with age-related diseases. In oocytes, telomeres of oocytes created after more rounds of mitosis may be shorter and could suffer further shortening due to reactive oxygen species, since telomerase is absent in oocytes[259].

Summary

Age at menopause has important effects on women's health, however relatively little is known about the biological processes that determine the end of female reproductive lifespan. Although approximately 50% of population variation in age at menopause is due to genetic causes, prior to the latest GWAS the genetic variants identified accounted for less than 3% of such variation. Further work is required to evaluate whether genes contributing to POI also impact on normal variation in menopause age. In the wider context of female reproduction, we also have limited understanding of the role of genetics in determining sex hormone levels, which are vital for the normal function of the female reproductive cycle and which change markedly around menopause. Non-genetic causes of menopause account for the remaining population variation in menopause age, but only smoking has consistently been found to have a strong effect, reducing age at menopause by 1–2 years and so further large studies are required to quantify the effect of other environmental risk factors. By combining the results of these complementary areas of research, we should be able to develop a more complete understanding of female reproductive ageing that ultimately may allow prediction of a woman's menopause age.

Table 3. Genes associated with age at menopause. (For full version of table see Appendix 3).

ANM=age at natural menopause; POI=primary ovarian insufficiency.

Gene	Type	Function of protein product	Location	Ref
ABAT	ANM	Catabolism of gamma-aminobutyric acid (GABA), a neurotransmitter in the central nervous system.	16p13.2	170
ACSL6	POI	Activation of long-chain fatty acids, role in fatty acid metabolism in brain.	5q31	245
ADAMTS19	POI	Releases extracellular domains of transmembrane proteins by proteolytic cleavage. Thought to play a role in gonad formation and function.	5q31	260, 261
AFF2	POI	Putative transcriptional activator. A repeat polymorphism in folate-sensitive fragile X E locus on chromosome X results in silencing of this gene causing Fragile X E syndrome, a form of nonsyndromic X-linked mental retardation.	Xq28	252, 253
ALOX12	POI	Lipid metabolism, generates bioactive lipid mediators that regulate processes such as platelet activation, angiogenesis, cell migration, proliferation.	17p13.1	262
AMH	POI	Part of transforming growth factor-beta superfamily. Involved in primary follicle formation, required for male sexual differentiation.	19p13.3-p13.2	207
AMHR2	ANM	Receptor for AMH.	12q13	263, 264, 169
ANKK1	ANM	Signal transduction, member of Ser/Thr protein kinase family.	11q23.2	176
APEX1	ANM	Cellular response to oxidative stress via DNA repair and regulation of transcription factors.	14q11.2	170
APOE	ANM	Mediates lipoprotein particle binding, internalization, and catabolism. ApoE ε4 linked to Alzheimer's disease, atherosclerosis.	19q13.31	265, 266
APTX	ANM	DNA-binding protein involved in DNA break repair and base excision repair.	9p13.3	170
AR	POI	Steroid hormone receptor. Ligand-activated transcription factor.	Xq11.2-q12	267, 268
ARHGEF7	ANM	Functions in cell migration, attachment and cell spreading.	13q34	166
ASCL1	ANM	Transcription factor. Role in early stages of development of CNS and peripheral nervous system.	4q35.1	170
BCAR4	ANM	Expressed in oocytes in cattle. Identified from screen of genes involved in tamoxifen resistance	16p13.13	168, 170
BCKDHB	POI	Catabolism of branched chain amino acids. Gene encodes E1 beta subunit - mutations in E1 cause maple syrup urine disease (MSUD), type 1B.	6q14.1	269
BMP15	ANM	Oocyte-specific growth/differentiation factor, stimulates folliculogenesis and granulosa cell growth	Xp11.22	264
	POI	Oocyte-specific growth/differentiation factor, stimulates folliculogenesis and granulosa cell growth	Xp11.22	188, 189, 190, 191
BRCA1	ANM	Part of complex involved in double strand break repair, tumour suppressor.	17q21.31	256, 170
BRCA2	ANM	Part of complex involved in double strand break repair, binds RAD51 recombinase	13q12-q13	256
BRE	ANM	Part of BRCA1-A complex, involved in double strand break damage repair.	2p23.3	168, 170
BRSK1	ANM	Polarization of neurons. Role in centrosome duplication via phosphorylation of gamma-tubulin. Part of UV-induced damage checkpoint response.	19q13.42	170; 165, 166, 167, 257, 169

Gene	Type	Function of protein product	Location	Ref
C16orf72	ANM	Unknown	16p13.2	170
CDK12	ANM	Phosphorylates RNA polymerase II (POLR2A), regulates transcription elongation. Required for RNA splicing. Involved in regulation of MAP kinase activity.	17q12	170
CDKN1B	POI	Regulator of cell cycle, involved in G1 arrest.	12p13.1-p12	240
CENPU	ANM	Component of nucleosome-associated complex. Important for assembly of kinetochore proteins, mitotic progression and chromosome segregation.	4q35.1	170
CHD7	ANM	Transcriptional coactivator for nuclear receptors, proposed to be a chromatin remodelling protein, DNA-dependent ATPase activity, binding A/T-rich DNA.	8q12.2	170
CHEK2	ANM	Required for checkpoint-mediated cell cycle arrest, activation of DNA repair and apoptosis in response to the presence of DNA double-strand breaks..	22q12.1	170
CITED2	POI	Transcriptional coactivator /corepressor. Role in sex determination and early gonad development, left-right patterning during embryogenesis, differentiation of adrenal cortex.	6q23.3	270
CLPP	POI	Component of a mitochondrial ATP-dependent proteolytic complex.	19p13.3	244
COX10	POI	Nuclear-encoded factor needed for assembly of cytochrome C oxidase, final enzyme in mitochondrial respiratory chain.	17p12	255
CPEB1	POI	RNA-binding protein, regulates mRNA cytoplasmic polyadenylation and translation initiation during oocyte maturation, early development and at post-synapse sites of neurons.	15q25.2	238
CYP19A1	ANM	Haem binding, oxido-reductase activity	15q21	176
	POI	Haem binding, oxido-reductase activity	15q21	219
CYP1B1	ANM	Metabolism of pro-carcinogens and 17beta-oestradiol.	2p22.2	271, 272, 273
CYP3A4	ANM	Metabolism of steroids and carcinogens.	7q22.1	273
DACH2	POI	Transcription factor, involved in regulation of organogenesis	Xq21.3	274
DAZL	POI	RNA-binding protein, essential for gametogenesis in both males and females. Role in spermatogenesis.	3p24	206
DDX17	ANM	RNA-dependent ATPase, transcriptional regulation, transcriptional coactivator for ESR1.	22q13.1	170
DIAPH2	POI	Member of diaphanous subfamily of the formin homology family of proteins. May play a role in the development and normal function of the ovaries.	Xq22	275
DIDO1	ANM	Cytoplasmic protein that translocates to the nucleus on apoptotic signal activation and is upregulated by apoptotic signals. Induces apoptosis.	20q13.33	170
DMC1	ANM	Meiotic recombination, specifically in resolving double-strand breaks.	22q13.1	170
	POI	Meiotic recombination, specifically in resolving double-strand breaks.	22q13.1	173
DMRT1	POI	Transcription factor, testis development and male germ cell proliferation. Represses transcription of female promoting genes, activates male-specific genes.	9p24.3	276
EIF2B2	POI	Subunit of eukaryotic initiation factor 2B (EIF2B), required for GTP exchange during protein synthesis.	14q24.3	277
EIF2B3	POI	Subunit of eukaryotic initiation factor 2B (EIF2B), required for GTP exchange during protein synthesis.	1p34.1	278, 279
EIF2B4	ANM	Subunit of eukaryotic initiation factor 2B (EIF2B), required for GTP exchange during protein synthesis.	2p23.3	176, 170
	POI	Subunit of eukaryotic initiation factor 2B (EIF2B), required for GTP exchange during protein synthesis.	2p23.3	277

Gene	Type	Function of protein product	Location	Ref
EIF2B5	POI	Subunit of eukaryotic initiation factor 2B (EIF2B), required for GTP exchange during protein synthesis.	3q27.1	277
EIF3M	ANM	Part of the eukaryotic translation initiation factor 3 (eIF-3) complex, required for initiation of protein synthesis.	11p13	170
eIF4ENIF1	POI	Nuclear import of EIF4E, shuttles between nucleus and cytoplasm	22q11.2	280
ESR1	ANM	Oestrogen receptor, ligand activated transcription factor which localises to nucleus and forms a dimer with oestrogen receptor 2.	6q25.1	281
	POI	Oestrogen receptor, ligand activated transcription factor which localises to nucleus and forms a dimer with oestrogen receptor 2.	6q25.1	282, 283, 284, 285, 286
EXO1	ANM	5' to 3' double-stranded DNA exonuclease, DNA mismatch repair. Endonuclease activity against 5'-overhanging flap structures.	1q43	168, 170
F5	ANM	Critical cofactor for the prothrombinase activity of factor Xa, regulator of homeostasis.	1q23	265
FAM175A	ANM	Component of the BRCA1-A complex. Involved in DNA damage response and double-strand break repair.	4q21.23	170
FANCA	POI	DNA repair. Part of complex composed of FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL/PHF9 and FANCM missing in Fanconi anaemia patients, who have cytogenetic instability, hypersensitivity to DNA crosslinking agents, increased chromosomal breakage, and defective DNA repair.	16q24.3	232
FANCI	ANM	Repair of DNA double-strand breaks by homologous recombination, repair of inter-strand DNA cross-links. Binds branched DNA.	15q26.1	176; 168, 170
FBXO18	ANM	ATP-dependent DNA helicase activity. Member of the F-box protein family.	10p15.1	170
FIGLA	POI	Germline specific transcription factor, oocyte-specific gene expression including those initiating folliculogenesis, may also be required for normal ovarian development in adults.	2p13.3	287, 288
FMR1	POI	Fragile X mental retardation 1 protein, RNA binding protein, expansion of CGG repeat in 5'UTR causes fragile X syndrome.	Xq27.3	Some recent citations: 181,289, 183, 290, 182, 180
FOXE1	POI	Thyroid transcription factor, thyroid gland organogenesis.	9q22	291, 292
FOXL2	POI	Transcriptional regulator, critical for ovary differentiation and maintenance, prevents differentiation of ovary to testis.	3q22.3	192, 193, 194, 195, 196, 197, 198
FOXO1A	POI	Transcription factor, target of insulin signalling, regulates metabolic homeostasis. Promotes neural cell death. Regulates adipogenic gene expression.	13q14.1	293
FOXO3	POI	Transcriptional activator, triggers apoptosis in the absence of survival factors.	6q21	293, 294
FSHB	ANM	Pituitary glycoprotein, encodes beta subunit of FSH, stimulates follicle development in ovary.	11p13	176, 168, 172, 170
	POI	Pituitary glycoprotein, encodes beta subunit of FSH, stimulates follicle development in ovary	11p13	177, 178, 179, 295
FSHR	POI	Receptor for FSH, G-protein receptor.	2p21-p16	214, 215, 216, 217, 218, 219, 220
GAD1	ANM	Catalyses production of the neurotransmitter GABA.	2q31.1	170

Gene	Type	Function of protein product	Location	Ref
GALT	POI	Catalyses galactose metabolism Absence of this enzyme results in classic galactosaemia.	9p13	296, 297
GDF9	POI	Required for ovarian folliculogenesis. Promotes primordial follicle development. Stimulates granulosa cell proliferation.	5q31.1	199, 200, 190, 201, 202,203
GSPT1	ANM	Translation termination in response to the codons UAA, UAG and UGA. Involved in regulation of mammalian cell growth.	16p13.13	168, 170
GTF3C2	ANM	Required for RNA polymerase-III mediated transcription. Part of TFIIIC that initiates transcription complex assembly on tRNA.	2p23.3	170
HARS2	POI	ATPase involved in DNA replication.	5q31.3	298
HAX1	POI	Promotes cell survival, cell migration, involved in clathrin-mediated endocytosis.	1q21.3	241
HDC	ANM	Catalyses the biosynthesis of histamine from histidine	15q21.2	299
HDX	POI	Homeobox protein, specific function unknown. Homeobox genes are involved in embryonic development	Xq21.1	300
HELB	ANM	Unwinds duplex DNA with 5' to 3' polarity. Has single-strand DNA-dependent ATPase and DNA helicase activities.	12q14.3	170
HELQ	ANM	Single-stranded DNA-dependent ATPase and DNA helicase.	4q21.23	168, 170
HFM1	POI	Homologous recombination during meiosis, required for cross-over formation and complete synapsis.	1p22.2	235
HLA genes	ANM	Antigen presentation, immune function	6p21.33	168, 172, 170,
	POI	Antigen presentation, immune function	6p21.33	174, 175
HSD17B4	POI	Part of the beta-oxidation pathway for fatty acids.	5q2	301
IGF1	ANM	Growth factor stimulating cellular proliferation and differentiation	12q23.2	176
IGF2R	ANM	Binds IGF2, binds phosphorylated lysosomal enzymes in the Golgi apparatus facilitating transport to lysosomes.	6q25.3	176, 261
INHA	POI	Inhibits secretion of FSH by the pituitary. Part of inhibin A and inhibin B.	2q35	208, 209, 210, 211, 212, 213
INHBA	POI	Inhibits secretion of FSH by the pituitary. Part of inhibin A.	7p15-p13	208
INO80	ANM	DNA helicase, component of the chromatin remodelling INO80 complex which is involved in transcriptional regulation, DNA replication and probably DNA repair.	15q15.1	170
KISS1R	ANM	Receptor for metastin, involved in suppression of metastasis. Regulation of the gonadotropic axis at puberty and in adulthood.	19p13.3	170
KNTC1	ANM	Stops cells from prematurely exiting mitosis, required for assembly of dynein-dynactin and MAD1-MAD2 complexes onto the kinetochores.	12q24.31	170
LAMC1	POI	Extracellular matrix glycoprotein. Role in cell adhesion, differentiation, migration, signalling.	1q31	302
LARS2	POI	Catalyses the aminoacylation of a specific tRNA	3p21.3	251
LEP	POI	Part of signalling pathway that regulates body fat stores.	7q31	303
LHB	POI	Reproductive hormone, stimulates testes and ovary to synthesise steroids.	19q13	304
LHCGR	ANM	Receptor for luteinizing hormone/choriogonadotropin.	2p21	176
	POI	Receptor for LH. G-protein coupled receptor.	2p21	305

Gene	Type	Function of protein product	Location	Ref
LMNA	POI	Part of the nuclear lamina, a fibrous layer on the nucleoplasmic side of the inner nuclear membrane. Role in nuclear assembly, chromatin organization, nuclear membrane and telomere dynamics.	1q22	306
MAK	ANM	Phosphorylates FZR1 in a cell cycle-dependent manner, role in the transcriptional co-activation of AR, suggested function in spermatogenesis.	6p24.2	165, 168, 170
MCM8	ANM	DNA helicase. Homologous recombination.	20p12.3	165, 166, 167, 168, 169, 170
	POI	DNA helicase. Homologous recombination.	20p12.3	171
MCM9	POI	DNA helicase, homologous recombination.	6q22.31	231
MSH5	ANM	Involved in meiotic recombination, needed for crossovers between homologs.	6p21.33	168, 172, 170
	POI	Involved in meiotic recombination, needed for crossovers between homologs.	6p21.3	173
MSH6	ANM	DNA mismatch repair, forms heterodimer with MSH2.	2p16.3	172, 170
MT-ATP6	POI	Mitochondrial membrane ATP synthase, produces ATP from ADP from a proton gradient across the mitochondrial membrane which is generated by electron-transport during respiration.	mt	307
MTHFR	ANM	catalyses the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate.	1p36.3	308
MYCBP	ANM	E3 ubiquitin-protein ligase. Possible role during synaptogenesis.	1p34.3	168, 169, 170
NANOS3	POI	Possible translational repressor. Regulates translation. Required for primordial germ cell development and maintenance in other organisms.	19p13.12	247, 248, 249
NBN	ANM	DNA damage response, member of the MRE11/RAD50 double-strand break repair complex, mutations are associated with Nijmegen breakage syndrome.	8q21-q24	176
	POI	DNA damage response, member of the MRE11/RAD50 double-strand break repair complex, mutations are associated with Nijmegen breakage syndrome.	8q21-q24	309
NLRP11	ANM	Involved in inflammation.	19q13.42	170; 165, 166, 167, 257, 169
NOBOX	POI	Transcription factor, essential for folliculogenesis and regulation of oocyte-specific genes in mice	7q35	204, 205
NOG	POI	Inhibits bone morphogenic protein (BMP) signalling.	17q22	310, 311
NR5A1	POI	Transcriptional regulator of genes involved in hypothalamic-pituitary-gonadal axis and adrenal axis	9q33	221, 222, 223, 224,225, 226, 227, 228, 229, 230
NXF5	POI	RNA binding protein implicated in mRNA nuclear export.	Xq22.1	312
PAI-1	POI	Serine protease inhibitor, suggested involvement in control of fibrinolysis.	7q22.1	313
PAPD7	ANM	Post-transcriptional quality control, poly(A) polymerase activity.	5p15.31	170
PARL	ANM	Control of apoptosis during post-natal growth.	3q27.1	170
PARP2	ANM	Involved in base excision repair pathway, catalyses poly(ADP-ribosyl)ation of acceptor proteins involved in chromatin architecture and in DNA metabolism following DNA damage.	14q11.2	170
PCSK1	ANM	Processes hormones at pairs of basic amino acids.	5q15-q21	176

Gene	Type	Function of protein product	Location	Ref
PGAP3	ANM	Involved in GPI anchor formation.	17q12	170
PGR	ANM	Progesterone receptor, involved in cellular differentiation and proliferation.	11q22-q23	176
PGRMC1	POI	Mediates anti-apoptotic action of progesterone in ovarian cells, regulator of steroid hormone biosynthesis.	Xq22-q24	314
PITPNM2	ANM	Catalyses the transfer of phosphatidylinositol and phosphatidylcholine between membranes.	12q24.31	170
PIWIL1	ANM	Central role during spermatogenesis by repressing transposable elements.	12q24.33	170
POF1B	POI	Regulates actin cytoskeleton, organisation of epithelial monolayers, possible role in ovary development.	Xq21	315
POLG	ANM	Replication of mitochondrial DNA	15q24	176; 168, 170
	POI	Replication of mitochondrial DNA	15q24	316, 317, 318
POLR2E	ANM	Component of DNA-dependent RNA polymerase. Part of lower jaw of RNA polymerase that attaches to incoming DNA template.	19p13.3	170
POLR2H	ANM	Component of DNA-dependent RNA polymerase.	3q27.1	170
POU5F1	POI	Transcription factors, controls genes involved in embryonic development.	6p21.31	254
PPARG	ANM	Activated by ligand to bind to DNA response elements, involved in adipocyte differentiation and glucose homeostasis, controls the peroxisomal beta-oxidation pathway of fatty acids.	3p25	176
PPARG	POI	Activated by ligand to bind to DNA response elements, involved in adipocyte differentiation and glucose homeostasis, controls the peroxisomal beta-oxidation pathway of fatty acids.	3p25	303
PPY	ANM	Regulates pancreatic and gastrointestinal functions, synthesised in pancreas.	14q11.2	170
PRIM1	ANM	Synthesises small RNA primers for Okazaki fragment synthesis during lagging strand DNA replication.	12q13.3	168, 169, 170
PSMC3IP	POI	Role in meiotic recombination, stimulates strand exchange .	17q21.2	236
PTHB1	POI	Thought to be involved in parathyroid hormone action in bones.	7p14	319
RAD51	ANM	DNA damage response, activation of homologous recombination and double strand break repair.	15q15.1	170
RAD54L	ANM	DNA repair, mitotic recombination (RAD52 pathway), possible role in telomere maintenance.	1p33	170
RET	POI	Receptor tyrosine-protein kinase, involved in cell growth, proliferation, differentiation.	10q11	242
REV3L	ANM	Part of DNA polymerase zeta, involved in trans-lesion DNA synthesis.	6q21	170
RHBDL2	ANM	Releases functional polypeptides from their membrane anchors, intramembrane proteolysis.	1p34.3	168, 257, 169, 170
RPAIN	ANM	Mediates import of RPA protein into the nucleus, which is required to stabilise single strand DNA formed during DNA replication or damage.	17p13.2	170
RPL10	POI	Subunit of ribosome responsible for protein synthesis.	Xq28	250
SALL4	POI	Transcription factor, maintenance and self-renewal of embryonic and hematopoietic stem cells.	20q13.13-q13.2	320
SETX	POI	Probable RNA/DNA helicase. Role in diverse aspects of RNA metabolism and genomic integrity. Essential for male meiosis.	9q34	233, 234
SH3PXD2B	ANM	Involved in invadopodia and podosome formation, cellular motility, role in adipocyte formation.	5q35.1	170

Gene	Type	Function of protein product	Location	Ref
SHBG	POI	Binds and transports 5-alpha-dihydrotestosterone, testosterone, and 17-beta-estradiol.	17p13	321
SLCO4A1	ANM	Sodium independent transport of organic anions, e.g. thyroid hormones, oestrone-3-sulphate.	20q13.33	170
SMAD7	ANM	Inhibits TGF-beta signalling, which controls proliferation and differentiation of cells.	18q21.1	176
SOHLH2	POI	Probable transcription factor, may be involved in spermatogenesis and oogenesis.	13q13.3	322
SPPL3	ANM	Aspartic protease, cleaves type II membrane signal peptides.	12q24.31	170
SRD5A1	ANM	Converts testosterone, progesterone and corticosterone into 5-alpha-3-oxosteroids	5p15.31	176
SRSF9	ANM	Constitutive splicing, selection of alternative splice sites.	12q24.31	170
STAG3	POI	Subunit of cohesin ring which ensures sister chromatid cohesion during meiosis	7q22.1	237
STAR	ANM	Steroid hormone synthesis, mediates the transfer of cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane where it is cleaved to pregnenolone.	8p12	168, 170
STARD3	ANM	Binds and transports cholesterol.	17q12	170
STX6	ANM	Intracellular vesicle trafficking.	1q25.3	170
SYCE1	POI	Component of synaptonemal complexes formed between homologous chromosomes during meiosis	10q26.3	238, 239
SYCP2L	ANM	Expressed in ovary.	6p24.2	165, 168, 172, 170
TAC3	ANM	Critical central regulator of gonad function.	12q13.3	168, 169, 170
TDRD3	ANM	Scaffold protein in nucleus and cytoplasm. Recognises asymmetric dimethylation associated with transcriptional activation.	13q21.2	168, 170
TGFBR1	ANM	Part of receptor for the TGF-beta cytokines TGFB1, TGFB2 and TGFB3, involved in cell cycle, proliferation, differentiation	9q22	176
TGFBR2	POI	Part of receptor for the TGF-beta cytokines TGFB1, TGFB2 and TGFB3, involved in cell cycle, proliferation, differentiation	3p22	323
TGFBR3	POI	Receptor which often functions as a co-receptor with other TGF-beta receptor superfamily members.	1p33-p32	243
TLK1	ANM	Involved in chromatin assembly processes.	2q31.1	168, 170
TNF	ANM	Cytokine that binds the receptors TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR to induce cell death.	6p21.3	176
U2AF2	ANM	Binds polypyrimidine tract of introns during spliceosome assembly.	19q13.42	170; 165, 166, 167, 169
UBE2MP1	ANM	Ubiquitin-conjugating (E2) enzyme pseudogene.	16p11.2	170
UIMC1	ANM	Binds 'Lys-63'-linked ubiquitinated histones H2A and H2AX at DNA lesion sites.	5q35.2	165, 168, 257, 169, 170
VEGFA	POI	Growth factor, increases endothelial cell proliferation, role in angiogenesis and vasculogenesis.	6p12	324
WNT4	POI	Involved in development. Ligand for members of the frizzled family of seven transmembrane receptors.	1p35	325
XPNPEP2	POI	Metalloprotease, role in inflammation and responses to injury and infection.	Xq26.1	246
ZNF729	ANM	May play role in transcriptional regulation.	19p12	170

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Chapter 2:

The influence of early life events on premature reproductive ageing: analysis of cross-sectional survey data from the UK Biobank

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Main text

Abstract

The available oocyte pool is determined before birth, with the majority of oocytes lost before puberty. We hypothesised that events occurring before birth, in childhood or in adolescence ('early-life risk factors') could influence the size of the oocyte pool and thus the timing of menopause. We included cross-sectional data from 273,474 women from the UK Biobank, recruited in 2006–2010 from across the UK. We analysed the association of early menopause with early-life risk factors in 11,781 cases (menopause aged under 45) and 173,641 controls (menopause/pre-menopausal at ≥45 years), in models controlling for potential confounding variables. Being part of a multiple birth was strongly associated with early menopause (odds ratio=1.42, confidence interval: 1.11, 1.82, P=8.0×10^{-9}, fully-adjusted model). Earlier age at menarche (odds ratio =1.03, confidence interval: 1.01, 1.06, P=2.5×10^{-6}) and earlier year of birth were also associated with EM (odds ratio=1.02, confidence interval: 1.00, 1.04, P=8.0×10^{-6}). We identified an association between multiple births and early menopause that has not previously been reported, which connects events pre-birth, when the oocyte pool is formed, with reproductive ageing in later life.

Introduction

Age at menopause influences health in later life with earlier menopause associated with increased osteoporosis and cardiovascular disease, and poorer cognitive function, but lower risks of several reproductive cancers [1,2]. Over half of the population variation in menopause age is estimated to be non-genetic, however only smoking and nulliparity have been reproducibly linked to earlier menopause [3].

Natural menopause occurs on average at 51 years of age in Caucasian populations when the number of oocytes in the ovary are reduced below about 1000, however the factors that determine the timing of this event are poorly understood [4]. The number of ovarian follicles is determined before birth: approximately 7 million oocytes are produced by 6 months post conception, though this number declines rapidly before birth, continuing after birth so that by puberty only about 400,000 primary oocytes remain. Over 99% of ovarian follicle loss is due to atresia [5], hence the rate of loss will influence age at menopause [3]. We hypothesised that pre-birth and early-life events could influence the oocyte pool and thus influence the timing of menopause. Early life events have shown inconsistent relationships with age at menopause [3]. Previously reported positive associations with menopause include birth weight [6-8], birth year [9], whether breast fed as a baby [10,11], food deprivation [12,13], age at menarche [14] childhood socio-economic status [15], maternal smoking during pregnancy [16], and weight in early childhood [6,10,11]. However, several studies have also reported null associations with these same traits, creating substantial uncertainty in the epidemiological literature [9,17-19].

In this, the largest epidemiological analysis of age at menopause to date, we present robust evidence for the association of early life variables with the clinically-relevant outcome of menopause below the age of 45 years (early menopause (EM)). In particular, that being part of a multiple birth and an earlier year of birth are associated with EM.

Methods

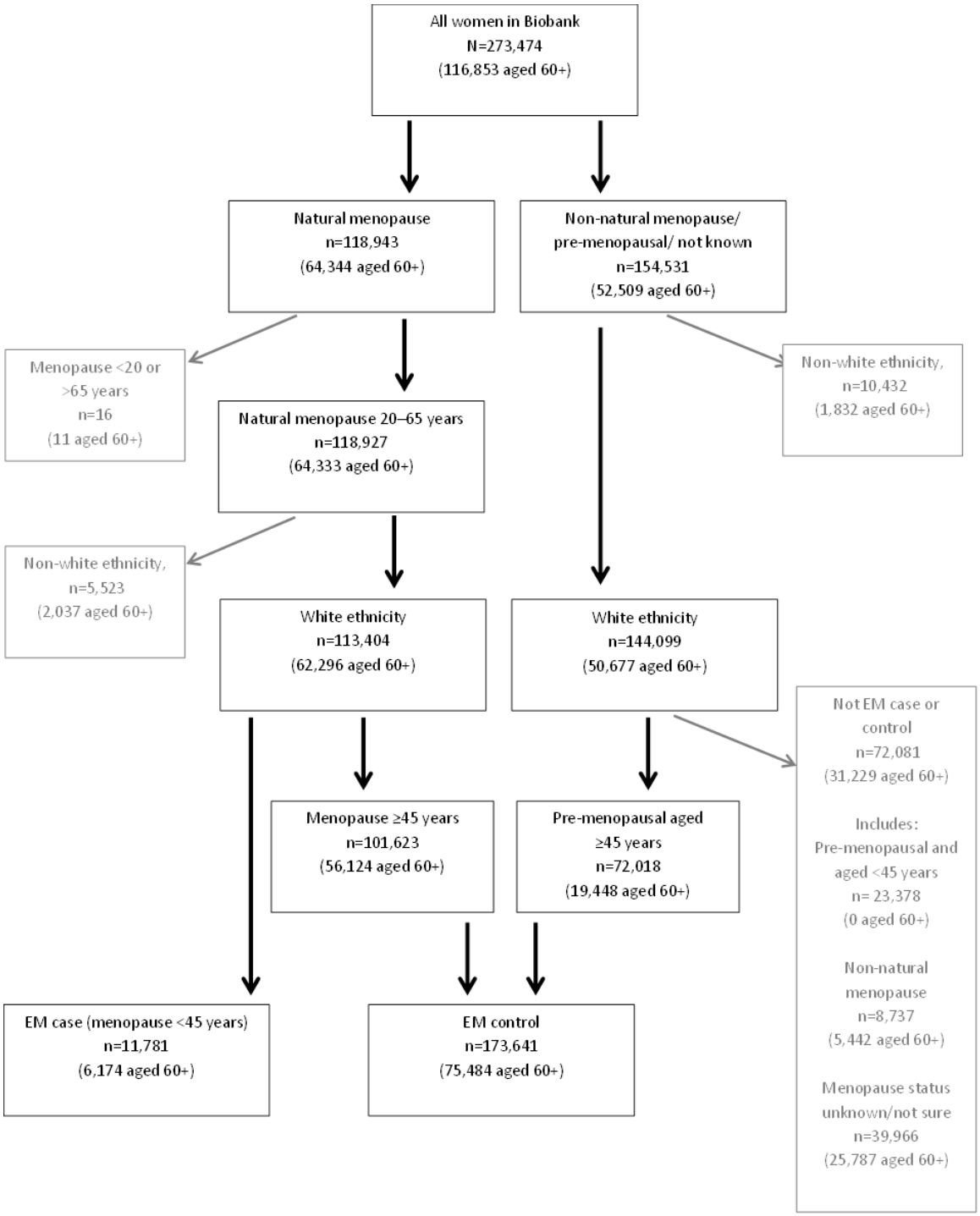
Source of data

We analysed data from 273,474 women from the UK Biobank, which includes 503,325 people aged 40–69 years recruited in 2006–2010 from across the UK [20] (further details in Supplementary Methods).

Identification of early menopause cases and controls

We considered the outcome EM in our analyses, since this is clinically relevant and captures the lower extreme of normal reproductive ageing. EM cases were women with menopause under 45 years while controls had menopause or were known to be pre-menopausal at age 45 or over. Age at natural menopause was defined as age at last menstrual period excluding those with surgical menopause or taking hormone replacement therapy . Of the 257,503 white women, there were 11,781 EM cases (4.6%) and 173,641 controls (67.4%) (Figure 1).

Figure 1. Selection of data used in the analysis.



Early life variables

We hypothesised that early-life risk factors influence the timing of menopause. Early-life risk factors have a clear temporal relationship with menopause and are suitable for analysis in cross-sectional data since they are unlikely to be biased by, or cause bias in, age at menopause. We defined an early-life risk factor as a risk-factor occurring before birth, in childhood or in adolescence. Fifteen early-life risk factors were present in the data: birth year, maternal smoking around birth, birth weight, part of a multiple birth, breastfed as a baby, adopted as a child, handedness, comparative height at age 10, comparative body size at age 10, age of menarche and five illnesses occurring at under 20 years-of-age. Illnesses diagnosed under the age of 20 were analysed in broad categories and were selected on the basis of frequency and relevance to reproductive phenotypes: 'allergy', 'infections', 'headaches', 'gynaecological issues' and 'cancer' (more details are provided in Supplementary Methods).

Statistical methods

We used logistic regression to conduct analyses of early-life variables and odds of EM (n included from 98,276 to 181,784). All analyses were performed in Stata/SE v13.1. We restricted our analyses to women of white ethnicity (95% of cohort) and removed extreme outliers as appropriate (see Supplementary Methods). Potential confounding variables were controlled for in two ways: Firstly, the 'partially-adjusted' model included variables associated with age at menopause in published studies that were significant in exploratory univariate analyses of our UK Biobank data (approximately 1000 variables tested), and included Townsend deprivation index, BMI and smoking status; Secondly, the 'fully-adjusted' model, included all variables significantly associated with menopause in exploratory univariate analyses, and included smoking pack-years, frequency of alcohol intake, number of live births, educational level and whether the participant ate meat. Due to the number of variables present in our UK Biobank data (approximately 1000), we used a conservative significance threshold of $P<5\times10^{-5}$ for all tests and calculated 99.995% confidence intervals (CIs) around the odds ratio (OR).

Consistency of relationships and sensitivity analyses

We performed parallel analysis using Cox proportional hazards regression models to check for consistency of relationships with menopause as a quantitative trait (Supplementary Methods) (n included from 126,001 to 228,221). To investigate the sensitivity of our results to age at recruitment which was different in cases and controls (Supplementary Fig. 1), we performed the same analyses in a subset of women aged 60 and over. Of 112,887 women aged 60 and over, there were 6,174 EM cases (5.5%) and 75,484 controls (66.9%) (Figure 1).

Results

Distribution of age at menopause is skewed

We identified 113,417 women with natural menopause, with a mean age at menopause of 50 years (Table 1) The distribution of age at menopause was skewed even when only women aged 60 and over were considered, and had peaks at values ending in zero, two and five. (Supplementary Fig. 2). Descriptive statistics for the cohort are presented in Table 2 and Supplementary Table 1.

Table 1. Age at natural menopause of women with self-reported white ethnicity in UK Biobank.

	n	*N*	%	Mean age at menopause in years (standard deviation)	Median age at menopause in years (range)
Natural menopause	113,417	257,516	44.0	50.0 (4.5)	50 (18,65)
Early menopause (<45 years)	11,794	233,394	5.1	40.6 (3.5)	42 (18,44)

Notes: n is number of women with natural menopause or early menopause. N is the total number of women for whom age at natural menopause (includes women with menopause at <20 years or >65 years) or early menopause status (women aged 45 and over at recruitment) could be determined.

Table 2. Descriptive statistics for early life variables.

		All cohort		Natural menopause		Early menopause (<45 years)	
Age of menarche	n	250,143		111,034		11,565	
	mean	13.0		13.0		12.9	
	s.d.	1.6		1.6		1.7	
	median (range)	13 (5,25)		13 (5,25)		13 (5,21)	
Birth weight (kg)	n	164,448		69,367		7,210	
	mean	3.2		3.2		3.2	
	s.d.	0.6		0.6		0.7	
	median (range)	3.2 (0.4,9)		3.2 (0.4,9)		3.2 (0.7,6.3)	
Year of birth	n	257,516		113,417		11,794	
	mean	1951.5		1948.4		1949.6	
	s.d.	8.0		5.6		7.1	
	median (range)	1950 (1936,1970)		1948 (1936,1969)		1948 (1936,1969)	
		n	%	n	%	n	%
Breastfed as a baby	No	64,388	31.0	23,563	26.1	2,807	30.1
	Yes	143,195	69.0	66,689	73.9	6,531	69.9
	Total	207,583		90,252		9,338	
Comparative height size at age 10	Shorter	53,619	21.2	23,549	21.1	2,502	21.6
	Taller	64,628	25.5	28,113	25.2	2,987	25.8
	Average	134,773	53.3	59,943	53.7	6,105	52.7
	Total	253,020		111,605		11,594	
Comparative body size at age 10	Thinner	79,326	31.3	34,402	30.7	3,890	33.5
	Plumper	45,203	17.8	19,358	17.3	2,050	17.6
	Average	129,271	50.9	58,170	52.0	5,685	48.9
	Total	253,800		111,930		11,625	
Maternal smoking around birth	No	156,728	70.3	70,228	71.6	6,977	68.2
	Yes	66,291	29.7	27,904	28.4	3,256	31.8
	Total	223,019		98,132		10,233	
Part of a multiple birth	No	247,777	97.7	109,292	97.6	11,242	96.9
	Yes	5,928	2.3	2,637	2.4	365	3.1
	Total	253,705		111,929		11,607	

Notes: Based on women with self-reported white ethnicity in UK Biobank.

Associations of potential confounding variables

Being a current smoker had the largest effect on EM (for current smokers compared with never smokers, OR=1.44, CI: 1.20, 1.73, $P<1\times10^{-15}$) (Figure 2) (Supplementary Tables 1–3). Other non-early life factors associated with earlier menopause were never drinking alcohol (e.g. OR=1.28, CI: 1.09, 1.51, $P=2.5\times10^{-10}$ for never drinking compared with drinking 1–2 times per week), decreasing educational level (OR=1.09, CI: 1.07, 1.12, $P<1\times10^{-15}$ per level), and fewer live births (OR=1.09, CI: 1.05, 1.13, $P<1\times10^{-15}$ per birth).

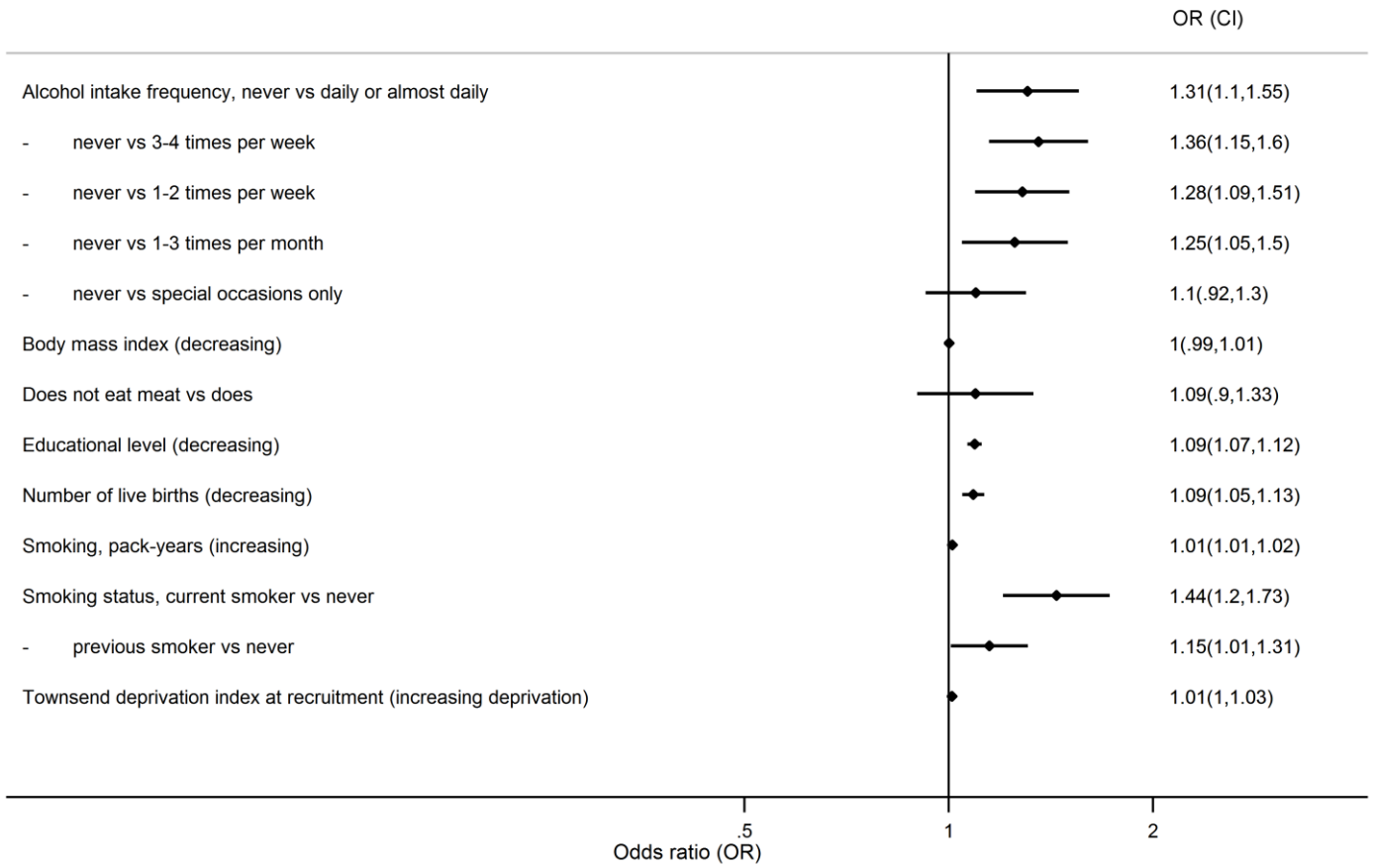
Events before birth are associated with EM

Earlier year of birth was associated with EM (OR=1.02, CI: 1.00, 1.04, $P=8\times10^{-6}$) in the partially- and fully-adjusted models, as was decreasing birth weight and being part of a multiple birth (Figure 3) (Supplementary Tables 4 and 5). For the effect of year of birth on EM to be as large as being a current smoker, a women would need to be born 16.9 years earlier (2.1 standard deviations).

Maternal smoking was significantly associated with EM only in the partially-adjusted model. The effect size for being part of a multiple birth (OR=1.42, CI: 1.11, 1.82, $P=8\times10^{-9}$, fully-adjusted model) was similar to that of being a current smoker (OR=1.44, CI: 1.20, 1.73, $P<1\times10^{-15}$, fully-adjusted model). These associations remained when multiple birth, decreasing birth weight and maternal smoking were considered in the same logistic regression model (Supplementary Table 6).

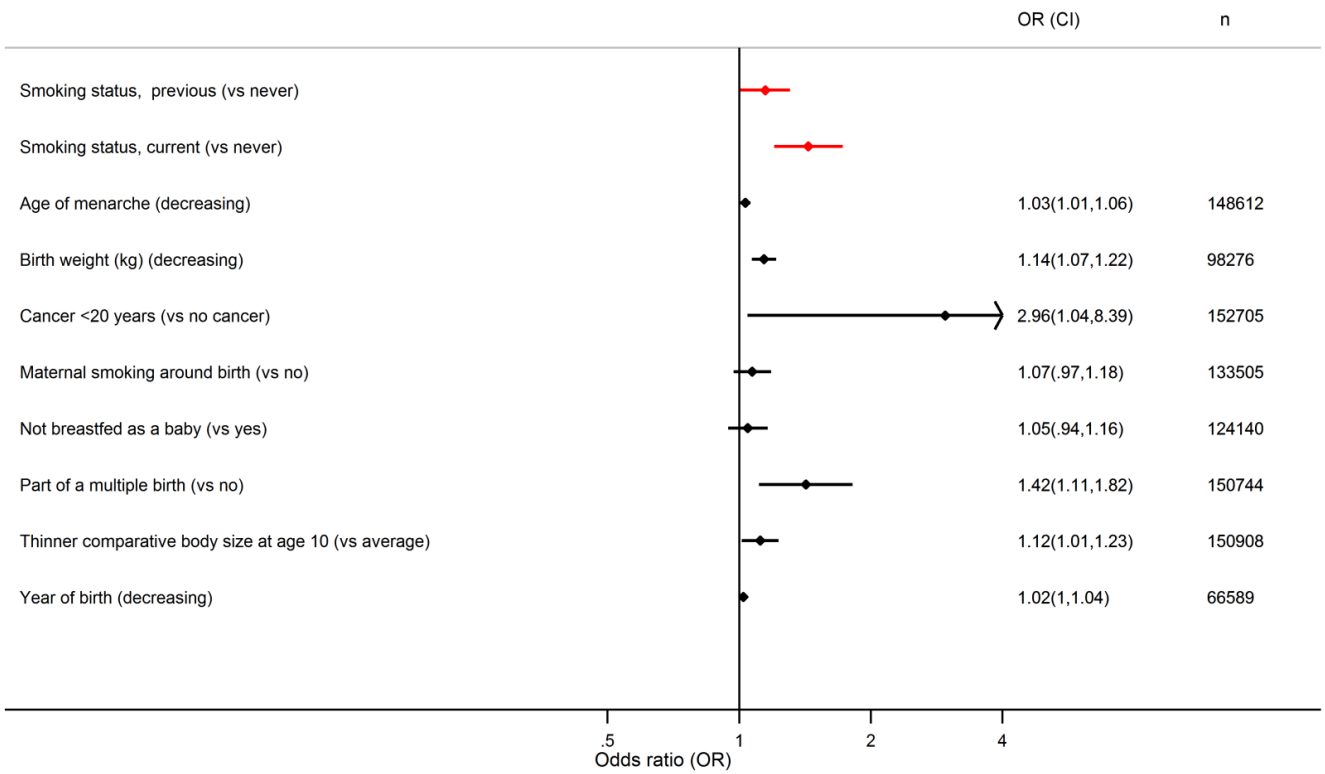
Since the association of EM with birth weight is adjusted for BMI, it could be considered that this is actually an association between EM and change in size [21]. The association with birth weight persisted regardless of adjustment for BMI (fully-adjusted model including BMI, OR=1.14 per kg, $P=2.5\times10^{-10}$; fully-adjusted model without BMI, OR=1.14 per kg, $P=2.3\times10^{-10}$), indicating that the association was with birth weight and not change in size.

Figure 2. Associations of potential confounding variables with early menopause.



Notes: Results shown presented are for the fully-adjusted logistic regression model including all potential confounding variables at the same time (n=152,705). Confidence intervals are 99.995%.

Figure 3. Associations between early-life risk factors and early menopause.



Notes: Results are for the fully-adjusted logistic regression model including one early-life risk factor at a time. Analyses are in all ages, except for year of birth which was analysed in women aged 60 and over at recruitment. Smoking status is included for reference. Confidence intervals are 99.995%. To achieve the same effect size as being a current smoker (the binary variable with nominally the largest effect size), for the continuous variables the required change in risk factor would be: age at menarche, 11.1 years (7.0 s.d.); birth weight 2.8kg (4.7 s.d.); year of birth, 16.9 years (2.1 s.d.).

Events in childhood and adolescence are associated with earlier menopause

Earlier age at menarche was associated with EM in the fully-adjusted logistic model (OR=1.03, CI: 1.01, 1.06, $P=2.5\times10^{-6}$), but not the partially-adjusted model (Figure 3, Supplementary Tables 2 and 3). The perception of being 'thinner at age 10' was significantly associated with EM in the partially- and fully-adjusted models (OR=1.12, CI: 1.01, 1.23, $P=3.5\times10^{-6}$ for fully-adjusted) (Figure 3). When comparative body size at age 10 was included with age at menarche in the model (Supplementary Table 7), both remained significantly associated with EM. The description of being 'plumper at age 10' was not associated with age at menopause in any of the models. Handedness, whether adopted as a child and being breastfed were not associated with EM (Figure 3) (Supplementary Tables 2–5).

EM is associated with cancer but not with other illnesses

Considering illnesses under the age of 20, only cancer was associated with EM (Figure 3, Supplementary Tables 2 and 3), but only in the fully-adjusted model (OR=2.96, CI: 1.04, 8.39, $P=2.4\times10^{-5}$). There were no significant associations with allergy, gynaecological problems, infections and headache/migraine.

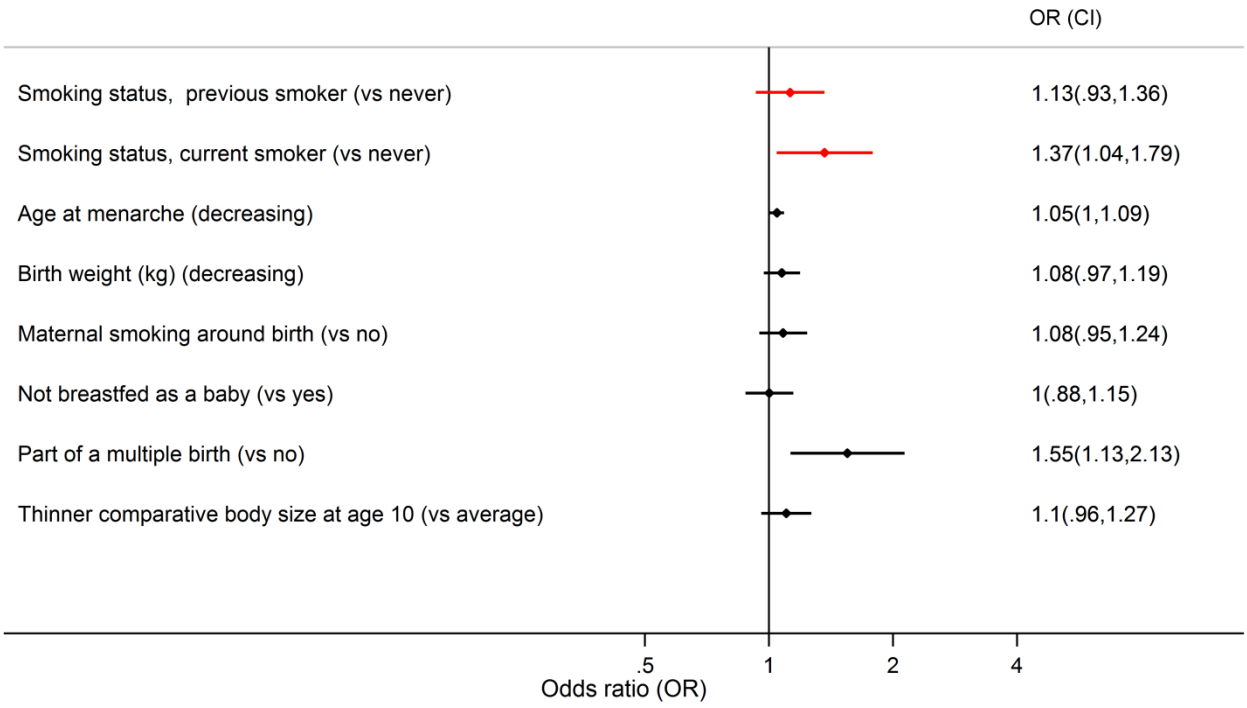
Consistency of relationships in Cox proportional hazards model of menopause as a quantitative trait and sensitivity analysis in the 60 and over age group

Using Cox proportional hazards regression, we were able to explore whether associations with EM also held for menopause as a quantitative trait, where the outcome is hazard ratio (HR) of menopause. The potential confounding variables that were significantly associated with EM – drinking alcohol, educational level, number of live births, and smoking – also had consistent directions of effect in the Cox model. Increasing social deprivation, lower BMI and not eating meat were associated with increased HR of menopause, though these risk factors were not associated with EM (Supplementary Tables 2–5) (Supplementary Fig. 3). The directions of effect for the early-life risk factors were consistent in the EM and Cox models (Supplementary Tables 2–5). In the analysis based on women aged 60 and over, the direction of effects was consistent with the analysis in all age groups (Supplementary Fig. 3).

Multiple birth and menarche remain strongly associated in models including all early-life risk factors

In a fully-adjusted model including all early-life risk factors that were associated with EM (age at menarche, birth weight, maternal smoking, breast fed as a baby, part of multiple birth and comparative body size at age 10), only the risk factors younger age at menarche (OR=1.05, CI: 1.0, 1.09, $P=7.27\times10^{-6}$) and being part of a multiple birth (OR=1.55, CI: 1.13, 2.13, $P=2.11\times10^{-8}$) remained significantly associated with EM (Figure 4). Cancer at under 20 years and year of birth were not included in the model including all early-life risk factors, due to the small number of cases in the former, and bias due to age at recruitment in the latter.

Figure 4. Associations in the multiple early-life variable model.



Notes: Results shown are for the fully-adjusted model in all age groups including all early-life risk factors at the same time (n=78,605). The early life risk-factors included are age at menarche, birth weight, maternal smoking, breast fed as a baby, part of multiple birth and comparative body size at age 10. Cancer at under 20 years and year of birth were not included due to the small number of cases in the former, and confounding with age at recruitment in the latter. Confidence intervals are 99.995%.

Discussion

Our study is the largest to date on the effect of non-genetic risk factors on age at menopause, compared with the previous largest studies in 95,704 US women and 50,678 UK women [14,22]. In this study, we have shown that pre-birth and early-life risk factors are associated with EM, an event in later life. Being part of a multiple birth demonstrated the most robust association, with an effect size comparable to being a smoker in this study, the single biggest predictor previously identified [9,14,17,18,22-24]. Non-early life risk factors are better characterised in the current literature than early-life variables and our results were consistent with previous studies. We found that odds of EM were increased by being a current smoker [9,14,17,18,22-24], having decreased levels of education [17,18,24,25] and being nulliparous [14,22]. However, we did not find consistent associations between age at menopause and BMI[14,22], socio-economic status and not eating meat [15,22,26], as suggested by previous studies.

Being part of a multiple birth and birth weight were the pre-birth risk factors with the strongest associations with EM. An association of EM with multiple births has not previously been reported, though the prevalence of premature ovarian failure has been found to be higher in twins[19,27]. The rate of multiple births in our study (2.4%) was slightly lower than recent estimates (3%), since the rate has increased in recent years due to assisted reproduction [8,28,29]. The association of multiple births with EM in this study should be independent of any potential effects of assisted reproduction since women in the study were born prior to its introduction. Babies from a multiple pregnancy are more likely to suffer complications including intrauterine growth restriction and are smaller at birth. However, intra-uterine growth restriction has not been found to restrict ovary growth and development [30]. Multiple births are more common as women age [29] and therefore the size of the oocyte pool could be reduced in offspring of older mothers. This could be mediated by genetic abnormalities, hypertension or birth complications, which are known to be more common in older women. However, we tested for effects of increased maternal age in our data and found no increased odds of EM, in fact there was a modest effect in the opposite direction, though we were unable to adjust for potential confounding factors such as maternal socio-economic status and age at menopause and survivor bias. In our study there was an association of EM with birth weight, however the

effect was less strong than that of multiple births but this may be because we were unable to adjust our analysis by gestational age. Evidence from other studies of the effects of birth weight has been contradictory [6,8,19,31].

The association between earlier age at menarche and increased odds of EM agrees with another analysis using the UK Biobank data [32], and is supported by findings from a study on over 90,000 women [14] and a smaller study on several thousand women [33] which both found associations between earlier menarche and earlier menopause. In addition, a published study has reported a correlation between genetic variants associated with age at menarche and age at menopause (r=0.138; *P*=0.04), though individual variants affecting both traits have not been found [34]. We found that being thinner at age 10 was associated with increased odds of EM. It is unlikely that this association is driven by an effect on menarche timing as being thinner in childhood is associated with delayed menarche [35,36] and in our data early menarche was a risk factor for EM. The role of childhood nutrition has been highlighted by previous studies that found associations between not being breast fed and earlier age at menopause [10,11] (though this was not significantly associated with EM in our study) and earlier menopause in women who are lighter at 1–2 years [6,10,11] or who have been exposed to famine in early childhood [37].

In our cohort of women born in 1936–1970, there was an increased odds of EM in women born in earlier birth years. We estimate an increase in age at menopause of about 1.3–2.5 months per year of later birth, which agrees with two smaller studies on cohorts born between 1908–1930 in Sweden (n=1,017) and 1912–1969 in the USA (n=22,851) [33,38]. The US study found a 17-month increase in age at menopause during 1915–1939 [38], while the Swedish study found an increase of 1.2 months per later birth year.

In our analysis of illnesses occurring before the age of 20, only cancer showed an association with EM. The illness data used were self-reported and may possibly be affected by recall bias [39,40]. By restricting these analyses to cases under 20 we have minimised uncertainty about cause and effect of the illness and menopause. However, compared to a prospective study of incident cases, our cohort will be biased since the women included will have had to have survived until the study. It is likely that cancer treatment will have had an effect

on age at menopause – such treatment is known to affect fertility [41] and has late side-effects such as cardiovascular disease and second primary cancers in later life [42,43].

A limitation of this study is that the UK Biobank data is based on volunteers and so there may be selection bias. The data used in our analysis was collected retrospectively and so may be subject to recall bias. Clear rounding of menopause age was evident, as has been seen in previous studies [44]. Previous studies have indicated that menopause is reasonably well recalled, though error increases with time after menopause [45,46]. Ethnicity has been shown to be associated with age at menopause [14,17,25] and we restricted our analysis to white women as we had insufficient numbers of women of other ethnicities to conduct sub-analyses. Age at menopause is limited by age at recruitment, however this should not confound the effect of variables other than year of birth, which was analysed in a post-menopausal cohort to address this. In addition there could be additional confounders that were not captured by our data. There was variation in the number of responses for each variable, with only about 40% of the case–control cohort included in the full analysis model with all early-life risk factors, and we cannot rule out that such missing-ness could be informative.

Due to the number of women included in this study, we provide powerful evidence for the influence of early-life risk factors on EM, and hence women's health. More specifically, that being a multiple birth, having menarche at a younger age, and being born in an earlier birth cohort are associated with EM . It is plausible that early-life events could influence the oocyte pool since the number of ovarian follicles is determined before birth and the number of primary oocytes decline dramatically prior to puberty – from about 7 million oocytes at 6 months post conception to about 400,000 oocytes by puberty. Early life events identified in our study could affect the oocyte reserve *in utero* or in early development and could be mediated through increased stress to oocytes, perhaps caused by reduced nutritional or oxygen availability. Factors affecting the number of follicles and their quality, and the rate of follicle decline both in utero and following birth could plausibly affect age at menopause, which itself is a consequence of a reduction in the follicle pool. At the cellular level, DNA repair has been identified as an important mechanism influencing age at

menopause and may result in maintenance of a larger, better quality follicle pool [47]. Since up to 50% of population variation in age at menopause is thought to be due to genetics, risk factors affecting the size of the follicle pool are likely to have different effects on age at menopause according to the genetic variants present. We anticipate that these finding should provide direction for further work into the biological processes driving menopause, and in particular how genetics and the environment interact.

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Author contributions

A.M. designed and directed the study, reviewed results of analysis and revised the manuscript. K.S.R. performed the statistical analysis and prepared the manuscript. W.E.H., D.M., M.N.W. and J.R.B.P advised on the analytic strategy, reviewed results of statistical analysis and commented on the manuscript.

Additional information

The authors declare no competing financial interests.

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Supplementary Methods

Source of data

UK Biobank invited 9.2 million people to participate, giving a response rate of 5.47%[20]. Participants were registered with the NHS and lived within 25 miles of one of the 22 assessment centres. Participants answered detailed questions about themselves, had measurements taken and provided samples. The data included follow-up data collected in 2012–2013 for 20,000 participants from North West England. In our analysis we analysed baseline data for 266,237 people with a single visit, and follow-up data for 7,237 participants.

Exclusion of outliers

Women with menopause under 20 or over 65 were excluded (n=16) on the basis of biological plausibility. Age at menarche was restricted to a clinically normal range of 9–17 years. Birth weights in excess of 6kg were excluded (n=86) as implausible/extreme outliers.

Cox proportional hazards model

In the Cox regression, the event was natural menopause and women were censored at bilateral oophorectomy and/or hysterectomy, or start of HRT use (where ongoing at time of menopause). The Cox proportional hazards assumption was tested using Schoenfeld residuals, however in most cases this did not hold.

Variables included

For further details of the variables included, please see http://www.ukbiobank.ac.uk/

Questions used to define menopause.

Age at menopause was derived from the following. Women providing no answer to any of the relevant questions, answering ‘Prefer not to answer’ or answering ‘Not sure’ were excluded from the analysis.

2724: Had menopause. Have you had your menopause (periods stopped)?

Prefer not to answer

No

Yes

Not sure - had a hysterectomy

Not sure - other reason

3581: Age at menopause. How old were you when your periods stopped?

3591: Ever had hysterectomy?

Not sure

Prefer not to answer

No

Yes

2824: Age at hysterectomy?

2834: Bilateral oophorectomy?

Not sure

Prefer not to answer

No

Yes

3882: Age at bilateral oophorectomy?

2814: Ever used HRT?

Prefer not to answer

Do not know

No

Yes

3536: Age started HRT?

3546: Age last used HRT?

Questions used to define the early life variables

Women providing no answer to any of the relevant questions, answering ‘Prefer not to answer’ or answering ‘Not sure’ were excluded from the analysis.

34: Year of birth

1787: Maternal smoking around birth

Prefer not to answer

Do not know

No

Yes

20022: Birth weight

1777: Part of a multiple birth

Prefer not to answer

Do not know

No

Yes

1677: Breastfed as a baby

Prefer not to answer

Do not know

No

Yes

1767: Adopted as a child

Prefer not to answer

Do not know

No

Yes

1707: Handedness (chirality/laterality)

Prefer not to answer

Right-handed

Left-handed

Use both right and left hands equally

1697: Comparative height at age 10

Prefer not to answer

Do not know

Shorter

Taller

About average

1687: Comparative body size at age 10

Prefer not to answer

Do not know

Thinner

Plumper

About average

2714: Age when periods started (menarche)

Questions used to define the potential confounding variables

Women providing no answer to any of the relevant questions, answering ‘Prefer not to answer’ or answering ‘Not sure’ were excluded from the analysis.

189: Townsend deprivation index at recruitment

1558: Alcohol intake frequency

Prefer not to answer

Daily or almost daily

Three or four times a week

Once or twice a week

One to three times a month

Special occasions only

Never

2734: Number of live births

20116: Smoking status

Prefer not to answer

Never

Previous

Current

21001: Body mass index (BMI)

Educational level was derived from:

6138: Qualifications

None of the above

Prefer not to answer

College or University degree

A levels/AS levels or equivalent

O levels/GCSEs or equivalent

CSEs or equivalent

NVQ or HND or HNC or equivalent

Other professional qualifications eg: nursing, teaching

Pack-years was derived using:

20116: Smoking status

Prefer not to answer

Never

Previous

Current

2867: Age started smoking in former smokers

2887: Number of cigarettes previously smoked daily

2897: Age stopped smoking

3436: Age started smoking in current smokers

3456: Number of cigarettes currently smoked daily (current cigarette smokers)

21003: Age at recruitment

Whether the participant ate meat was derived from:

1359: Poultry intake

Prefer not to answer

Do not know

Never

Less than once a week

Once a week

2-4 times a week

5-6 times a week

Once or more daily

1369: Beef intake

Prefer not to answer

Do not know

Never

Less than once a week

Once a week

2-4 times a week

5-6 times a week

Once or more daily

1379: Lamb/mutton intake

Prefer not to answer

Do not know

Never

Less than once a week

Once a week

2-4 times a week

5-6 times a week

Once or more daily

1389: Pork intake

Prefer not to answer

Do not know

Never

Less than once a week

Once a week

2-4 times a week

5-6 times a week

Once or more daily

Illnesses included (all have at least one case at under 20 years)

Cancer aged under 20 was identified from ‘20007: Interpolated age of participant when cancer first diagnosed’. Other illnesses were identified from ‘20002: Non-cancer illness code, self-reported’ and ‘20009: Interpolated age of participant when non-cancer illness first diagnosed’.

Allergy N=20,391	Cancer N=298	Gynaecological problems N=736	Infections N=13,792	Migraine/ headache N=4,906
Allergy or anaphylactic reaction to drug	Any self-reported cancer reported under 20 years:	Abnormal smear (cervix)	Acute infective polyneuritis/ Guillain-Barre syndrome	Migraine
Allergy or anaphylactic reaction to food	Acute myeloid leukaemia	Cervical erosion	Appendicitis	Headaches (not migraine)
Allergy to house dust mite	Basal cell carcinoma	Cervical polyps	Blepharitis/ eyelid infection	
Allergy/ hypersensitivity/ anaphylaxis	Bladder cancer	Cervical problem	Bronchitis	
Asthma	Brain cancer / primary malignant brain tumour	Dysmenorrhoea / dysmenorrhea	Cellulitis	
Blistering/ desquamating skin disorder	Breast cancer	Ectopic pregnancy	Chickenpox	
Contact dermatitis	Cancer of lip/ mouth/ pharynx/ oral cavity	Female infertility	Dengue fever	
Crohn's disease	Cervical cancer	Fibrocystic disease	Diphtheria	
Eczema/ dermatitis	Chronic lymphocytic	Gynaecological disorder (not cancer)	Encephalitis	
Food intolerance	Chronic myeloid	Menopausal symptoms / menopause	Gastroenteritis/dysentery	
Hay fever/ allergic rhinitis	CIN/ pre-cancer cells cervix	Menorrhagia (unknown cause)	Helicobacter pylori	
Psoriasis	Colon cancer/ sigmoid cancer	Miscarriage	Hepatitis	
Ulcerative colitis	Ear cancer	Ovarian cyst or cysts	Hepatitis A	
	Eye and/ or adnexal cancer	Ovarian problem	Hepatitis B	
	Female genital tract cancer	Pelvic inflammatory disease/PID	Hepatitis C	
	Hodgkin's lymphoma / Hodgkin's disease	Polycystic ovaries/ polycystic ovarian syndrome	Herpes simplex	
	Kidney/ renal cell cancer	Uterine fibroids	Infection of nervous system	
	Large bowel cancer/ colorectal cancer	Uterine polyps	Infectious mononucleosis/ glandular fever/ Epstein Barr virus (EBV)	
	Leukaemia	Vaginal prolapse/ uterine prolapse	Infective/ viral hepatitis	
	Liver/ hepatocellular cancer		Lung abscess	
	Lung cancer		Malaria	
	Malignant lymph node, unspecified		Measles/ morbillivirus	
	Malignant melanoma		Meningitis	
	Meningeal cancer / malignant meningioma		Mumps/ epidemic parotitis	
	Non-Hodgkin's lymphoma		Non-infective hepatitis	
	Non-melanoma skin cancer		Pericarditis	
	Ovarian cancer		Peritonitis	
	Parotid gland cancer		Pneumonia	
	Peripheral nerve/ autonomic nerve cancer		Polio/ poliomyelitis	
	Primary bone cancer		Rheumatic fever	
	Retinoblastoma		Rubella/ German measles	
	Sarcoma/ fibrosarcoma		Scarlet fever/ scarlatina	
	Skin cancer		Schistosomiasis/ bilharzia	
	Small intestine/ small bowel cancer		Septicaemia/ sepsis	
	Spinal cord or cranial nerve cancer		Shingles	
	Thyroid cancer		Tonsillitis	
	Unclassifiable		Tuberculosis (TB)	
	Uterine/ endometrial cancer		Typhoid fever	
	Vaginal cancer		Whooping cough/ pertussis	
			Yellow fever	

Supplementary Results

Supplementary Table 1. Descriptive statistics for potential confounding variables (women with self-reported white ethnicity only).

		All cohort		Natural menopause		Menopause <45 years	
Age when attended assessment centre	n	257,516		113,417		11,794	
	mean	56.7		59.9		58.6	
	s.d.	8		5.6		7.1	
	median (range)	58 (39,73)		60 (40,73)		60 (40,73)	
Townsend deprivation index at recruitment	n	257,212		113,300		11,781	
	mean	-1.5		-1.6		-1.2	
	s.d.	2.9		2.9		3.1	
	median (range)	-2.3 (-6.3,11)		-2.3 (-6.3,10.6)		-2.1 (-6.3,10.2)	
Body mass index (BMI)	n	253,085		111,551		11,562	
	mean	27		26.9		27.1	
	s.d.	5.1		5		5.2	
	median (range)	26.1 (12.8,67.3)		26 (13.8,66.2)		26.3 (13.8,64.8)	
Amount smoked, pack-years	n	218,427		96,434		10,107	
	mean	6.4		6.7		10.1	
	s.d.	13		13.3		16.2	
	median (range)	0 (0,215)		0 (0,205)		0 (0,129)	
Number of live births	n	257,309		113,360		11,788	
	mean	1.8		1.8		1.7	
	s.d.	1.2		1.2		1.2	
	median (range)	2 (0,22)		2 (0,22)		2 (0,22)	
		n	**%**	**n**	**%**	**n**	**%**
Smoking status	never	150,413	59	66,085	59	5,942	51
	previous	83,215	32	37,902	34	4,231	36
	current	22,978	9	9,067	8	1,578	13
	Total	256,606		113,054		11,751	
Alcohol intake frequency	daily or almost daily	42,719	17	20,249	18	1,953	17
	3 or 4 times a week	54,677	21	24,054	21	2,258	19
	once or twice a week	67,974	26	29,065	26	2,954	25
	1 to 3 times a month	33,823	13	13,973	12	1,484	13
	special occasions	37,025	14	16,353	14	1,923	16
	never	21,128	8.2	9,677	8.5	1,216	10
	Total	257,346		113,371		11,788	
Highest educational level achieved	None	43,831	17	21,748	19	2,767	24
	CSEs	10,171	4	3,591	3.2	434	3.7
	GCSEs/O-levels	40,025	16	17,925	16	1,812	16
	A-levels / NVQ /HND /HNC	41,672	16	16,331	15	1,817	16
	Professional qual.	38,667	15	17,533	16	1,792	15
	degree	79,228	31	34,710	31	2,984	26
	Total	253,594		111,838		11,606	
Eats poultry, beef, lamb/mutton or pork	yes	244,420	95	107,807	95	11,176	95
	no	12,944	5	5,556	4.9	613	5.2
	Total	257,364		113,363		11,789	

Supplementary Table 2. Partially-adjusted models in all ages of women at recruitment.

				Terms included in addition to variables i	
		Partially adjusted model		Adopted	
Risk factor included in model	**Statistic**	**Cox**	**Logistic**	**Cox**	**Logistic**
Townsend deprivation index at	HR/OR	1.010	1.027	1.010	1.027
recruitment (increasing deprivation)	*P*	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.001	0.003	0.001	0.003
Body mass index (BMI) (increasing)	HR/OR	0.990	1.008	0.990	1.008
	P	<1.0E-15	3.3E-05	<1.0E-15	4.5E-05
	s.e.	0.001	0.002	0.001	0.002
Smoking status, previous	HR/OR	1.082	1.306	1.082	1.305
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.007	0.028	0.007	0.028
Smoking status, current	HR/OR	1.303	1.949	1.304	1.947
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.015	0.060	0.015	0.060
Adopted as a child, yes	HR/OR			0.957	1.126
	P			1.1E-01	1.4E-01
	s.e.			0.026	0.091
r^2/pseudo-r^2		0.000	0.008	0.000	0.008
n		228,221	181,784	227,970	181,597

		Age at menarche		Birthweight	
Risk factor included in model	**Statistic**	**Cox**	**Logistic**	**Cox**	**Logistic**
Townsend deprivation index at	HR/OR	1.011	1.028	1.011	1.022
recruitment (increasing deprivation)	*P*	<1.0E-15	<1.0E-15	<1.0E-15	1.9E-07
	s.e.	0.001	0.003	0.001	0.004
Body mass index (BMI) (increasing)	HR/OR	0.990	1.007	0.990	1.007
	P	<1.0E-15	6.4E-04	<1.0E-15	2.5E-03
	s.e.	0.001	0.002	0.001	0.002
Smoking status, previous	HR/OR	1.081	1.305	1.079	1.324
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.007	0.028	0.009	0.036
Smoking status, current	HR/OR	1.304	1.950	1.322	2.037
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.015	0.061	0.020	0.080
Age of menarche (decreasing)	HR/OR	1.012	1.024		
	P	6.9E-10	2.6E-04		
	s.e.	0.002	0.007		
Birth weight (kg)	HR/OR			1.031	1.162
	P			3.1E-07	5.3E-15
	s.e.			0.006	0.022
r^2/pseudo-r^2		0.000	0.008	0.001	0.009
n		222,046	176,908	149,659	116,651

Risk factor included in model	Statistic	Breast fed		Comparative body size as child	
		Cox	Logistic	Cox	Logistic
Townsend deprivation index at recruitment (increasing deprivation)	HR/OR	1.011	1.026	1.010	1.025
	P	<1.0E-15	3.1E-12	<1.0E-15	2.9E-14
	s.e.	0.001	0.004	0.001	0.003
Body mass index (BMI) (increasing)	HR/OR	0.991	1.008	0.991	1.010
	P	<1.0E-15	2.7E-04	<1.0E-15	8.1E-07
	s.e.	0.001	0.002	0.001	0.002
Smoking status, previous	HR/OR	1.077	1.282	1.082	1.307
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.008	0.030	0.007	0.028
Smoking status, current	HR/OR	1.306	1.923	1.307	1.958
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.017	0.067	0.015	0.061
Breastfed as a baby, no	HR/OR	0.991	1.059		
	P	2.6E-01	1.6E-02		
	s.e.	0.008	0.022		
Comparative body size at age 10, plumper	HR/OR			0.987	0.989
	P			1.2E-01	7.0E-01
	s.e.			0.008	0.027
Comparative body size at age 10, thinner	HR/OR			1.011	1.144
	P			1.1E-01	7.2E-10
	s.e.			0.007	0.025
r^2/pseudo-r^2		0.000	0.007	0.000	0.008
n		187,069	147,747	225,523	179,604

Risk factor included in model	Statistic	Comparative height as child		Handedness	
		Cox	Logistic	Cox	Logistic
Townsend deprivation index at recruitment (increasing deprivation)	HR/OR	1.010	1.026	1.010	1.026
	P	<1.0E-15	6.0E-15	<1.0E-15	1.1E-15
	s.e.	0.001	0.003	0.001	0.003
Body mass index (BMI) (increasing)	HR/OR	0.990	1.007	0.990	1.008
	P	<1.0E-15	8.7E-05	<1.0E-15	4.1E-05
	s.e.	0.001	0.002	0.001	0.002
Smoking status, previous	HR/OR	1.082	1.304	1.082	1.307
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.007	0.028	0.007	0.028
Smoking status, current	HR/OR	1.304	1.959	1.303	1.949
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.015	0.061	0.015	0.060
Comparative height size at age 10, shorter	HR/OR	0.974	1.033		
	P	7.1E-04	1.9E-01		
	s.e.	0.008	0.026		
Comparative height size at age 10, taller	HR/OR	0.995	1.019		
	P	5.1E-01	4.3E-01		
	s.e.	0.007	0.024		
Handedness, both equally	HR/OR			1.009	1.210
	P			7.4E-01	1.6E-02
	s.e.			0.027	0.096
Handedness, left handed	HR/OR			0.979	0.941
	P			5.3E-02	8.3E-02
	s.e.			0.011	0.033
r^2/pseudo-r^2		0.000	0.008	0.000	0.008
n		224,905	179,141	228,199	181,768

Risk factor included in model	Statistic	Maternal smoking		Multiple birth	
		Cox	Logistic	Cox	Logistic
Townsend deprivation index at recruitment (increasing deprivation)	HR/OR	1.010	1.024	1.010	1.026
	P	<1.0E-15	1.6E-11	<1.0E-15	1.2E-14
	s.e.	0.001	0.004	0.001	0.003
Body mass index (BMI) (increasing)	HR/OR	0.991	1.008	0.990	1.008
	P	<1.0E-15	2.0E-04	<1.0E-15	2.2E-05
	s.e.	0.001	0.002	0.001	0.002
Smoking status, previous	HR/OR	1.080	1.298	1.081	1.299
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.008	0.029	0.007	0.028
Smoking status, current	HR/OR	1.314	1.938	1.301	1.940
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.017	0.065	0.015	0.060
Maternal smoking around birth, yes	HR/OR	1.019	1.114		
	P	8.4E-03	1.5E-06		
	s.e.	0.007	0.025		
Part of a multiple birth, yes	HR/OR			1.110	1.419
	P			1.7E-07	6.0E-10
	s.e.			0.022	0.080
r^2/pseudo-r^2		0.000	0.008	0.000	0.008
n		199,101	158,249	224,950	179,404

Risk factor included in model	Statistic	Allergy <20 year		Cancer <20 years	
		Cox	Logistic	Cox	Logistic
Townsend deprivation index at recruitment (increasing deprivation)	HR/OR	1.010	1.027	1.010	1.027
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.001	0.003	0.001	0.003
Body mass index (BMI) (increasing)	HR/OR	0.990	1.008	0.990	1.008
	P	<1.0E-15	3.2E-05	<1.0E-15	3.3E-05
	s.e.	0.001	0.002	0.001	0.002
Smoking status, previous	HR/OR	1.082	1.305	1.082	1.305
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.007	0.028	0.007	0.028
Smoking status, current	HR/OR	1.302	1.948	1.303	1.948
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.015	0.060	0.015	0.060
Allergy <20 years	HR/OR	0.948	0.940		
	P	6.6E-05	1.3E-01		
	s.e.	0.013	0.039		
Cancer <20 years	HR/OR			1.057	2.633
	P			6.7E-01	7.7E-05
	s.e.			0.138	0.645
r^2/pseudo-r^2		0.000	0.008	0.000	0.008
n		228,221	181,784	228,221	181,784

Risk factor included in model	Statistic	Gynecological problems <20 years		Headache <20 years	
		Cox	Logistic	Cox	Logistic
Townsend deprivation index at recruitment (increasing deprivation)	HR/OR	1.010	1.027	1.010	1.027
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.001	0.003	0.001	0.003
Body mass index (BMI) (increasing)	HR/OR	0.990	1.008	0.990	1.008
	P	<1.0E-15	3.3E-05	<1.0E-15	3.4E-05
	s.e.	0.001	0.002	0.001	0.002
Smoking status, previous	HR/OR	1.082	1.306	1.082	1.306
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.007	0.028	0.007	0.028
Smoking status, current	HR/OR	1.303	1.950	1.303	1.949
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.015	0.060	0.015	0.060
Gynecological problems <20 years	HR/OR	0.879	0.797		
	P	8.2E-02	3.2E-01		
	s.e.	0.065	0.184		
Headache <20 years	HR/OR			0.941	0.946
	P			1.2E-02	4.7E-01
	s.e.			0.023	0.072
r^2/pseudo-r^2		0.000	0.008	0.000	0.008
n		228,221	181,784	228,221	181,784

Risk factor included in model	Statistic	Infection <20 years	
		Cox	Logistic
Townsend deprivation index at recruitment (increasing deprivation)	HR/OR	1.010	1.026
	P	<1.0E-15	1.1E-15
	s.e.	0.001	0.003
Body mass index (BMI) (increasing)	HR/OR	0.990	1.008
	P	<1.0E-15	3.3E-05
	s.e.	0.001	0.002
Smoking status, previous	HR/OR	1.082	1.306
	P	<1.0E-15	<1.0E-15
	s.e.	0.007	0.028
Smoking status, current	HR/OR	1.303	1.950
	P	<1.0E-15	<1.0E-15
	s.e.	0.015	0.060
Infection <20 years	HR/OR	0.999	1.068
	P	9.6E-01	1.7E-01
	s.e.	0.015	0.051
r^2/pseudo-r^2		0.000	0.008
n		228,221	181,784

Supplementary Table 3. Fully-adjusted models in all ages of women at recruitment.

Risk factor included in model	Statistic	Terms included in addition to variables included in the fully adjusted model									
		Fully adjusted model		Adopted		Age of menarche		Birthweight		Breast fed	
		Cox	Logistic	Cox	Logistic	Cox	Logistic	Cox	Logistic	Cox	Logistic
Amount smoked, pack-years (increasing)	HR/OR	1.005	1.013	1.005	1.013	1.005	1.012	1.005	1.014	1.005	1.014
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.000	0.001	0.000	0.001	0.000	0.001	0.001	0.001	0.000	0.001
Alcohol intake frequency, daily or almost daily	HR/OR	0.928	0.765	0.928	0.764	0.927	0.764	0.915	0.811	0.936	0.785
	P	6.3E-08	4.4E-10	5.0E-08	3.6E-10	6.4E-08	5.8E-10	5.7E-07	1.5E-04	2.4E-05	6.0E-07
	s.e.	0.013	0.033	0.013	0.033	0.013	0.033	0.016	0.045	0.015	0.038
Alcohol intake frequency, three or four times a week	HR/OR	0.922	0.738	0.922	0.737	0.921	0.731	0.914	0.752	0.931	0.752
	P	1.0E-09	2.4E-13	8.5E-10	2.3E-13	9.7E-10	1.3E-13	1.2E-07	1.3E-07	2.4E-06	1.5E-09
	s.e.	0.012	0.031	0.012	0.031	0.012	0.031	0.016	0.041	0.014	0.035
Alcohol intake frequency, once or twice a week	HR/OR	0.937	0.779	0.936	0.778	0.934	0.780	0.931	0.777	0.947	0.785
	P	4.5E-07	2.5E-10	3.0E-07	2.0E-10	1.7E-07	5.6E-10	1.4E-05	9.5E-07	2.0E-04	6.3E-08
	s.e.	0.012	0.031	0.012	0.031	0.012	0.031	0.015	0.040	0.014	0.035
Alcohol intake frequency, one to three times a month	HR/OR	0.944	0.799	0.943	0.798	0.939	0.802	0.932	0.850	0.957	0.843
	P	6.4E-05	4.2E-07	4.9E-05	3.7E-07	1.4E-05	9.4E-07	1.5E-04	4.2E-03	6.6E-03	6.3E-04
	s.e.	0.014	0.035	0.014	0.035	0.014	0.036	0.017	0.048	0.016	0.042
Alcohol intake frequency, special occasions only	HR/OR	0.988	0.912	0.988	0.912	0.986	0.920	0.979	0.938	1.001	0.938
	P	3.9E-01	2.9E-02	3.8E-01	2.9E-02	3.3E-01	5.0E-02	2.4E-01	2.4E-01	9.6E-01	1.8E-01
	s.e.	0.014	0.038	0.014	0.038	0.014	0.039	0.018	0.052	0.016	0.045
Number of live births (increasing)	HR/OR	0.959	0.921	0.959	0.921	0.958	0.920	0.953	0.908	0.955	0.913
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.003	0.008	0.003	0.008	0.003	0.009	0.003	0.011	0.003	0.009
Highest educational level achieved (increasing)	HR/OR	0.980	0.916	0.980	0.916	0.979	0.914	0.979	0.916	0.979	0.908
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.002	0.005	0.002	0.005	0.002	0.006	0.002	0.007	0.002	0.006
Does not eat poultry, beef, lamb/mutton or pork	HR/OR	1.076	1.094	1.077	1.097	1.076	1.105	1.080	1.084	1.076	1.139
	P	2.2E-06	6.4E-02	1.8E-06	5.8E-02	2.9E-06	4.1E-02	7.2E-05	1.8E-01	1.9E-05	1.4E-02
	s.e.	0.017	0.053	0.017	0.053	0.017	0.054	0.021	0.066	0.018	0.060
Townsend deprivation index at recruitment (increasing deprivation)	HR/OR	1.005	1.011	1.005	1.011	1.006	1.012	1.006	1.007	1.006	1.009
	P	3.0E-05	3.9E-03	2.6E-05	3.5E-03	3.8E-06	1.4E-03	1.0E-04	1.3E-01	6.4E-06	2.2E-02
	s.e.	0.001	0.004	0.001	0.004	0.001	0.004	0.002	0.005	0.001	0.004
Body mass index (BMI) (increasing)	HR/OR	0.988	0.999	0.988	0.999	0.988	0.997	0.988	0.999	0.989	0.999
	P	<1.0E-15	5.4E-01	<1.0E-15	4.8E-01	<1.0E-15	1.8E-01	<1.0E-15	7.4E-01	<1.0E-15	5.7E-01
	s.e.	0.001	0.002	0.001	0.002	0.001	0.002	0.001	0.003	0.001	0.002
Smoking status, previous	HR/OR	1.036	1.147	1.036	1.147	1.035	1.157	1.034	1.148	1.032	1.119
	P	6.9E-04	2.0E-05	7.9E-04	2.0E-05	1.0E-03	8.1E-06	1.5E-02	9.6E-04	7.9E-03	1.9E-03
	s.e.	0.011	0.037	0.011	0.037	0.011	0.038	0.014	0.048	0.012	0.040
Smoking status, current	HR/OR	1.186	1.440	1.187	1.440	1.190	1.458	1.191	1.458	1.187	1.401
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	2.4E-15	8.3E-11	<1.0E-15	2.1E-11
	s.e.	0.020	0.064	0.020	0.064	0.020	0.066	0.026	0.085	0.022	0.070
Adopted as a child, yes	HR/OR			0.930	1.140						
	P			1.6E-02	1.3E-01						
	s.e.			0.028	0.100						
Age of menarche (decreasing)	HR/OR					1.014	1.033				
	P					9.5E-11	2.5E-06				
	s.e.					0.002	0.007				
Birth weight (kg) (decreasing)	HR/OR							1.029	1.140		
	P							1.1E-05	2.5E-10		
	s.e.							0.006	0.018		
Breastfed as a baby, no	HR/OR									0.992	1.047
	P									3.4E-01	7.5E-02
	s.e.									0.008	0.027

Risk factor included in model	Statistic	Comparative body size as child		Comparative height as child		Handedness		Maternal smoking	
		Cox	Logistic	Cox	Logistic	Cox	Logistic	Cox	Logistic
Amount smoked, pack-years (increasing)	HR/OR	1.005	1.013	1.005	1.012	1.005	1.013	1.006	1.013
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.000	0.001	0.000	0.001	0.000	0.001	0.000	0.001
Alcohol intake frequency, daily or almost daily	HR/OR	0.926	0.768	0.928	0.775	0.928	0.766	0.922	0.755
	P	2.4E-08	9.9E-10	6.0E-08	4.1E-09	6.2E-08	5.6E-10	3.3E-08	8.6E-10
	s.e.	0.013	0.033	0.013	0.034	0.013	0.033	0.014	0.035
Alcohol intake frequency, three or four times a week	HR/OR	0.919	0.740	0.922	0.746	0.922	0.739	0.918	0.732
	P	3.5E-10	6.8E-13	1.2E-09	3.2E-12	1.1E-09	3.9E-13	2.0E-09	2.1E-12
	s.e.	0.012	0.031	0.012	0.031	0.012	0.031	0.013	0.032
Alcohol intake frequency, once or twice a week	HR/OR	0.934	0.777	0.937	0.790	0.937	0.781	0.941	0.766
	P	1.1E-07	2.0E-10	4.2E-07	3.2E-09	4.5E-07	3.6E-10	8.9E-06	2.5E-10
	s.e.	0.012	0.031	0.012	0.031	0.012	0.031	0.013	0.032
Alcohol intake frequency, one to three times a month	HR/OR	0.941	0.799	0.942	0.806	0.944	0.801	0.947	0.805
	P	2.3E-05	5.2E-07	3.9E-05	1.4E-06	6.3E-05	5.4E-07	3.6E-04	4.1E-06
	s.e.	0.014	0.036	0.014	0.036	0.014	0.035	0.015	0.038
Alcohol intake frequency, special occasions only	HR/OR	0.984	0.910	0.988	0.927	0.988	0.913	0.990	0.913
	P	2.5E-01	2.7E-02	3.7E-01	7.6E-02	3.9E-01	3.0E-02	5.2E-01	4.2E-02
	s.e.	0.014	0.039	0.014	0.039	0.014	0.038	0.015	0.041
Number of live births (increasing)	HR/OR	0.958	0.920	0.959	0.917	0.959	0.921	0.957	0.913
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.003	0.008	0.003	0.008	0.003	0.008	0.003	0.009
Highest educational level achieved (increasing)	HR/OR	0.980	0.917	0.980	0.915	0.980	0.916	0.981	0.912
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.002	0.006	0.002	0.006	0.002	0.005	0.002	0.006
Does not eat poultry, beef, lamb/mutton or pork	HR/OR	1.076	1.113	1.075	1.094	1.076	1.093	1.077	1.145
	P	2.2E-06	2.9E-02	3.5E-06	6.7E-02	2.1E-06	6.6E-02	7.5E-06	7.9E-03
	s.e.	0.017	0.054	0.017	0.054	0.017	0.053	0.018	0.058
Townsend deprivation index at recruitment (increasing deprivation)	HR/OR	1.005	1.009	1.005	1.010	1.005	1.010	1.005	1.007
	P	8.5E-05	1.2E-02	5.1E-05	7.9E-03	3.1E-05	4.0E-03	2.4E-05	6.3E-02
	s.e.	0.001	0.004	0.001	0.004	0.001	0.004	0.001	0.004
Body mass index (BMI) (increasing)	HR/OR	0.989	1.000	0.988	0.998	0.988	0.999	0.989	0.998
	P	<1.0E-15	8.6E-01	<1.0E-15	4.6E-01	<1.0E-15	5.2E-01	<1.0E-15	4.5E-01
	s.e.	0.001	0.002	0.001	0.002	0.001	0.002	0.001	0.002
Smoking status, previous	HR/OR	1.034	1.140	1.036	1.148	1.036	1.149	1.029	1.132
	P	1.4E-03	5.1E-05	7.9E-04	2.1E-05	6.6E-04	1.7E-05	1.3E-02	3.6E-04
	s.e.	0.011	0.037	0.011	0.037	0.011	0.037	0.012	0.039
Smoking status, current	HR/OR	1.188	1.444	1.187	1.458	1.186	1.442	1.181	1.404
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	3.4E-12
	s.e.	0.020	0.065	0.020	0.066	0.020	0.064	0.022	0.068
Comparative body size at age 10, plumper	HR/OR	0.980	0.982						
	P	2.8E-02	5.4E-01						
	s.e.	0.009	0.029						
Comparative body size at age 10, thinner	HR/OR	1.007	1.117						
	P	3.3E-01	3.5E-06						
	s.e.	0.008	0.027						
Comparative height size at age 10, shorter	HR/OR			0.973	1.032				
	P			1.2E-03	2.5E-01				
	s.e.			0.008	0.028				
Comparative height size at age 10, taller	HR/OR			0.998	1.046				
	P			8.4E-01	8.3E-02				
	s.e.			0.008	0.027				
Handedness, both equally	HR/OR					1.015	1.238		
	P					6.1E-01	1.3E-02		
	s.e.					0.029	0.106		
Handedness, left handed	HR/OR					0.981	0.937		
	P					1.1E-01	9.2E-02		
	s.e.					0.012	0.036		
Maternal smoking around birth, yes	HR/OR							1.006	1.072
	P							4.3E-01	4.4E-03
	s.e.							0.008	0.026
r^2/pseudo-r^2		0.001	0.019	0.001	0.018	0.001	0.018	0.001	0.019
n		189,315	150,908	188,837	150,535	191,531	152,693	167,836	133,505

Risk factor included in model	Statistic	Multiple birth		Allergy <20 year		Cancer <20 years	
		Cox	Logistic	Cox	Logistic	Cox	Logistic
Amount smoked, pack-years (increasing)	HR/OR	1.005	1.012	1.005	1.013	1.005	1.013
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.000	0.001	0.000	0.001	0.000	0.001
Alcohol intake frequency, daily or almost daily	HR/OR	0.928	0.769	0.928	0.765	0.928	0.766
	P	7.5E-08	1.2E-09	6.4E-08	4.4E-10	6.4E-08	4.9E-10
	s.e.	0.013	0.033	0.013	0.033	0.013	0.033
Alcohol intake frequency, three or four times a week	HR/OR	0.922	0.738	0.922	0.738	0.922	0.738
	P	1.3E-09	4.7E-13	1.1E-09	2.4E-13	1.1E-09	3.0E-13
	s.e.	0.012	0.031	0.012	0.031	0.012	0.031
Alcohol intake frequency, once or twice a week	HR/OR	0.938	0.779	0.937	0.779	0.937	0.780
	P	7.0E-07	3.3E-10	4.6E-07	2.5E-10	4.5E-07	2.9E-10
	s.e.	0.012	0.031	0.012	0.031	0.012	0.031
Alcohol intake frequency, one to three times a month	HR/OR	0.945	0.801	0.944	0.799	0.944	0.800
	P	1.0E-04	7.0E-07	6.8E-05	4.2E-07	6.5E-05	4.8E-07
	s.e.	0.014	0.036	0.014	0.035	0.014	0.035
Alcohol intake frequency, special occasions only	HR/OR	0.987	0.913	0.988	0.912	0.988	0.913
	P	3.5E-01	3.2E-02	3.9E-01	2.9E-02	3.9E-01	3.0E-02
	s.e.	0.014	0.039	0.014	0.038	0.014	0.038
Number of live births (increasing)	HR/OR	0.959	0.921	0.959	0.920	0.959	0.921
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.003	0.008	0.003	0.008	0.003	0.008
Highest educational level achieved (increasing)	HR/OR	0.980	0.916	0.980	0.916	0.980	0.916
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.002	0.006	0.002	0.005	0.002	0.005
Does not eat poultry, beef, lamb/mutton or pork	HR/OR	1.077	1.099	1.076	1.094	1.076	1.093
	P	2.1E-06	5.5E-02	1.9E-06	6.4E-02	2.1E-06	6.8E-02
	s.e.	0.017	0.054	0.017	0.053	0.017	0.053
Townsend deprivation index at recruitment (increasing deprivation)	HR/OR	1.005	1.010	1.005	1.011	1.005	1.011
	P	5.4E-05	8.1E-03	3.1E-05	3.9E-03	3.0E-05	3.9E-03
	s.e.	0.001	0.004	0.001	0.004	0.001	0.004
Body mass index (BMI) (increasing)	HR/OR	0.988	0.999	0.988	0.999	0.988	0.999
	P	<1.0E-15	7.0E-01	<1.0E-15	5.4E-01	<1.0E-15	5.5E-01
	s.e.	0.001	0.002	0.001	0.002	0.001	0.002
Smoking status, previous	HR/OR	1.034	1.141	1.036	1.147	1.036	1.147
	P	1.7E-03	5.2E-05	7.1E-04	2.1E-05	7.0E-04	2.0E-05
	s.e.	0.011	0.037	0.011	0.037	0.011	0.037
Smoking status, current	HR/OR	1.182	1.439	1.186	1.440	1.186	1.440
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.020	0.065	0.020	0.064	0.020	0.064
Part of a multiple birth, yes	HR/OR	1.100	1.420				
	P	1.0E-05	8.0E-09				
	s.e.	0.024	0.086				
Allergy <20 years	HR/OR			0.964	0.996		
	P			1.1E-02	9.4E-01		
	s.e.			0.014	0.045		
Cancer <20 years	HR/OR					1.072	2.960
	P					6.3E-01	2.4E-05
	s.e.					0.155	0.760
r^2/pseudo-r^2		0.001	0.018	0.001	0.018	0.001	0.018
n		188,872	150,744	191,549	152,705	191,549	152,705

Risk factor included in model	Statistic	Gynecological problems <20 years		Headache <20 years		Infection <20 years	
		Cox	Logistic	Cox	Logistic	Cox	Logistic
Amount smoked, pack-years (increasing)	HR/OR	1.005	1.013	1.005	1.013	1.005	1.013
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.000	0.001	0.000	0.001	0.000	0.001
Alcohol intake frequency, daily or almost daily	HR/OR	0.928	0.765	0.927	0.765	0.928	0.765
	P	6.4E-08	4.4E-10	4.1E-08	4.5E-10	6.3E-08	4.5E-10
	s.e.	0.013	0.033	0.013	0.033	0.013	0.033
Alcohol intake frequency, three or four times a week	HR/OR	0.922	0.737	0.921	0.738	0.922	0.738
	P	1.0E-09	2.3E-13	6.5E-10	2.5E-13	1.0E-09	2.5E-13
	s.e.	0.012	0.031	0.012	0.031	0.012	0.031
Alcohol intake frequency, once or twice a week	HR/OR	0.937	0.779	0.936	0.779	0.937	0.780
	P	4.4E-07	2.4E-10	3.1E-07	2.6E-10	4.5E-07	2.6E-10
	s.e.	0.012	0.031	0.012	0.031	0.012	0.031
Alcohol intake frequency, one to three times a month	HR/OR	0.944	0.799	0.944	0.799	0.944	0.799
	P	6.3E-05	4.2E-07	5.4E-05	4.2E-07	6.4E-05	4.3E-07
	s.e.	0.014	0.035	0.014	0.035	0.014	0.035
Alcohol intake frequency, special occasions only	HR/OR	0.988	0.912	0.988	0.912	0.988	0.912
	P	3.9E-01	2.9E-02	3.7E-01	2.9E-02	3.8E-01	2.8E-02
	s.e.	0.014	0.038	0.014	0.038	0.014	0.038
Number of live births (increasing)	HR/OR	0.959	0.920	0.959	0.921	0.959	0.921
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.003	0.008	0.003	0.008	0.003	0.008
Highest educational level achieved (increasing)	HR/OR	0.980	0.916	0.980	0.916	0.980	0.916
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.002	0.005	0.002	0.005	0.002	0.005
Does not eat poultry, beef, lamb/mutton or pork	HR/OR	1.076	1.094	1.076	1.094	1.076	1.094
	P	1.9E-06	6.4E-02	2.0E-06	6.4E-02	2.1E-06	6.4E-02
	s.e.	0.017	0.053	0.017	0.053	0.017	0.053
Townsend deprivation index at recruitment (increasing deprivation)	HR/OR	1.005	1.011	1.005	1.011	1.005	1.010
	P	3.1E-05	3.9E-03	3.3E-05	3.9E-03	3.0E-05	4.1E-03
	s.e.	0.001	0.004	0.001	0.004	0.001	0.004
Body mass index (BMI) (increasing)	HR/OR	0.988	0.999	0.988	0.999	0.988	0.999
	P	<1.0E-15	5.4E-01	<1.0E-15	5.4E-01	<1.0E-15	5.4E-01
	s.e.	0.001	0.002	0.001	0.002	0.001	0.002
Smoking status, previous	HR/OR	1.036	1.147	1.036	1.147	1.036	1.147
	P	6.6E-04	2.0E-05	6.5E-04	2.0E-05	6.9E-04	2.1E-05
	s.e.	0.011	0.037	0.011	0.037	0.011	0.037
Smoking status, current	HR/OR	1.186	1.441	1.186	1.440	1.186	1.441
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.020	0.064	0.020	0.064	0.020	0.064
Gynecological problems <20 years	HR/OR	0.841	0.752				
	P	3.3E-02	2.7E-01				
	s.e.	0.068	0.194				
Headache <20 years	HR/OR			0.942	1.003		
	P			2.1E-02	9.7E-01		
	s.e.			0.025	0.081		
Infection <20 years	HR/OR					1.005	1.098
	P					7.8E-01	7.6E-02
	s.e.					0.016	0.058
r^2/pseudo-r^2		0.001	0.018	0.001	0.018	0.001	0.018
n		191,549	152,705	191,549	152,705	191,549	152,705

Supplementary Table 4. Partially adjusted models in women aged 60 and over at recruitment.

Risk factor included in model	Statistic	Partially adjusted mode		Terms included in addition to variables included in the partially adjuste: Adopted		Age at menarche		Birthweight	
		Cox	Logistic	Cox	Logistic	Cox	Logistic	Cox	Logistic
Townsend deprivation index at recruitment (increasing deprivation)	HR/OR	1.008	1.031	1.008	1.032	1.009	1.033	1.010	1.029
	P	1.1E-08	2.0E-11	1.1E-08	1.8E-11	2.5E-10	5.6E-12	1.5E-07	5.3E-06
	s.e.	0.001	0.005	0.001	0.005	0.001	0.005	0.002	0.007
Body mass index (BMI) (increasing)	HR/OR	0.990	1.012	0.989	1.012	0.989	1.011	0.989	1.012
	P	<1.0E-15	1.9E-05	<1.0E-15	1.9E-05	<1.0E-15	5.2E-05	<1.0E-15	9.5E-04
	s.e.	0.001	0.003	0.001	0.003	0.001	0.003	0.001	0.004
Smoking status, previous	HR/OR	1.067	1.290	1.068	1.290	1.066	1.292	1.056	1.306
	P	5.2E-14	<1.0E-15	5.3E-14	<1.0E-15	2.7E-13	<1.0E-15	2.7E-06	5.4E-12
	s.e.	0.009	0.037	0.009	0.037	0.009	0.037	0.012	0.051
Smoking status, current	HR/OR	1.203	1.859	1.205	1.860	1.209	1.866	1.212	1.878
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.020	0.089	0.021	0.089	0.021	0.091	0.029	0.126
Adopted as a child, yes	HR/OR			0.957	1.045				
	P			2.1E-01	6.9E-01				
	s.e.			0.034	0.116				
Age of menarche (decreasing)	HR/OR					1.008	1.004		
	P					4.3E-03	6.4E-01		
	s.e.					0.003	0.009		
Birth weight (kg) (decreasing)	HR/OR							1.022	1.166
	P							7.3E-03	1.4E-08
	s.e.							0.008	0.032
r^2/pseudo-r^2		0.000	0.007	0.000	0.007	0.000	0.007	0.000	0.008
n		92,122	79,889	92,012	79,791	90,034	78,043	52,236	45,153

Risk factor included in model	Statistic	Breast fed		Comparative body size as child		Comparative height as child		Handedness	
		Cox	Logistic	Cox	Logistic	Cox	Logistic	Cox	Logistic
Townsend deprivation index at recruitment (increasing deprivation)	HR/OR	1.010	1.029	1.008	1.031	1.008	1.030	1.008	1.031
	P	1.3E-08	1.3E-07	2.4E-08	1.0E-10	9.8E-09	3.7E-10	9.7E-09	2.0E-11
	s.e.	0.002	0.006	0.001	0.005	0.001	0.005	0.001	0.005
Body mass index (BMI) (increasing)	HR/OR	0.990	1.012	0.990	1.012	0.989	1.011	0.990	1.012
	P	<1.0E-15	1.8E-04	<1.0E-15	1.0E-05	<1.0E-15	6.0E-05	<1.0E-15	2.0E-05
	s.e.	0.001	0.003	0.001	0.003	0.001	0.003	0.001	0.003
Smoking status, previous	HR/OR	1.058	1.275	1.067	1.290	1.067	1.294	1.067	1.291
	P	1.4E-08	2.0E-13	1.2E-13	<1.0E-15	1.7E-13	<1.0E-15	5.2E-14	<1.0E-15
	s.e.	0.011	0.042	0.009	0.037	0.009	0.037	0.009	0.037
Smoking status, current	HR/OR	1.193	1.812	1.209	1.890	1.205	1.873	1.203	1.859
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.024	0.102	0.021	0.091	0.021	0.091	0.020	0.089
Breastfed as a baby, no	HR/OR	0.996	1.064						
	P	7.3E-01	8.9E-02						
	s.e.	0.011	0.039						
Comparative body size at age 10, plumper	HR/OR			0.985	1.061				
	P			2.0E-01	1.3E-01				
	s.e.			0.012	0.041				
Comparative body size at age 10, thinner	HR/OR			0.998	1.178				
	P			8.5E-01	6.5E-08				
	s.e.			0.009	0.036				
Comparative height size at age 10, shorter	HR/OR					0.983	1.044		
	P					1.0E-01	2.2E-01		
	s.e.					0.010	0.036		
Comparative height size at age 10, taller	HR/OR					1.000	1.014		
	P					9.9E-01	6.7E-01		
	s.e.					0.010	0.033		
Handedness, both equally	HR/OR							0.967	1.089
	P							3.6E-01	4.6E-01
	s.e.							0.035	0.125
Handedness, left handed	HR/OR							0.995	0.920
	P							7.4E-01	1.0E-01
	s.e.							0.015	0.047
r^2/pseudo-r^2		0.000	0.006	0.000	0.008	0.000	0.007	0.000	0.007
n		69,924	60,745	90,813	78,755	90,561	78,540	92,111	79,880

Risk factor included in model	Statistic	Maternal smoking		Multiple birth		Year of birth		Allergy <20 year		Cancer <20 years	
		Cox	Logistic	Cox	Logistic	Cox	Logistic	Cox	Logistic	Cox	Logistic
Townsend deprivation index at recruitment (increasing deprivation)	HR/OR	1.009	1.030	1.008	1.031	1.008	1.031	1.008	1.031	1.008	1.031
	P	3.2E-08	4.7E-09	1.2E-08	4.0E-11	2.2E-08	3.3E-11	1.1E-08	2.0E-11	1.1E-08	2.0E-11
	s.e.	0.002	0.005	0.001	0.005	0.001	0.005	0.001	0.005	0.001	0.005
Body mass index (BMI) (increasing)	HR/OR	0.990	1.011	0.990	1.012	0.989	1.011	0.990	1.012	0.990	1.012
	P	<1.0E-15	1.7E-04	<1.0E-15	1.9E-05	<1.0E-15	2.8E-05	<1.0E-15	1.9E-05	<1.0E-15	1.9E-05
	s.e.	0.001	0.003	0.001	0.003	0.001	0.003	0.001	0.003	0.001	0.003
Smoking status, previous	HR/OR	1.065	1.274	1.066	1.287	1.068	1.291	1.067	1.289	1.067	1.290
	P	2.9E-11	5.1E-15	2.6E-13	<1.0E-15	4.7E-14	<1.0E-15	5.4E-14	<1.0E-15	5.2E-14	<1.0E-15
	s.e.	0.010	0.039	0.009	0.037	0.009	0.037	0.009	0.037	0.009	0.037
Smoking status, current	HR/OR	1.217	1.838	1.200	1.854	1.204	1.869	1.202	1.856	1.203	1.858
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.023	0.097	0.021	0.090	0.020	0.090	0.020	0.089	0.020	0.089
Maternal smoking around birth, yes	HR/OR	1.006	1.143								
	P	5.6E-01	3.3E-05								
	s.e.	0.010	0.037								
Part of a multiple birth, yes	HR/OR			1.095	1.534						
	P			8.4E-04	3.9E-08						
	s.e.			0.030	0.120						
Year of birth (decreasing)	HR/OR					1.008	1.028				
	P					4.2E-09	3.8E-10				
	s.e.					0.001	0.004				
Allergy <20 years	HR/OR							0.973	0.920		
	P							1.6E-01	2.1E-01		
	s.e.							0.019	0.061		
Cancer <20 years	HR/OR									1.072	1.532
	P									7.3E-01	4.2E-01
	s.e.									0.219	0.815
r^2/pseudo-r^2		0.000	0.007	0.000	0.007	0.000	0.008	0.000	0.007	0.000	0.007
n		78,751	68,359	90,711	78,740	92,122	79,889	92,122	79,889	92,122	79,889

Risk factor included in model	Statistic	Gynecological problems <20 years		Headache <20 years		Infection <20 years	
		Cox	Logistic	Cox	Logistic	Cox	Logistic
Townsend deprivation index at recruitment (increasing deprivation)	HR/OR	1.008	1.031	1.008	1.031	1.008	1.031
	P	1.1E-08	2.0E-11	1.3E-08	2.2E-11	1.1E-08	2.1E-11
	s.e.	0.001	0.005	0.001	0.005	0.001	0.005
Body mass index (BMI) (increasing)	HR/OR	0.990	1.012	0.990	1.012	0.990	1.012
	P	<1.0E-15	1.9E-05	<1.0E-15	2.0E-05	<1.0E-15	1.9E-05
	s.e.	0.001	0.003	0.001	0.003	0.001	0.003
Smoking status, previous	HR/OR	1.067	1.290	1.068	1.290	1.067	1.290
	P	5.2E-14	<1.0E-15	5.1E-14	<1.0E-15	5.5E-14	<1.0E-15
	s.e.	0.009	0.037	0.009	0.037	0.009	0.037
Smoking status, current	HR/OR	1.203	1.859	1.202	1.856	1.203	1.859
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.020	0.089	0.020	0.089	0.020	0.089
Gynecological problems <20 years	HR/OR	0.944	0.700				
	P	5.7E-01	3.3E-01				
	s.e.	0.095	0.255				
Headache <20 years	HR/OR			0.926	0.772		
	P			2.6E-02	4.3E-02		
	s.e.			0.032	0.099		
Infection <20 years	HR/OR					0.983	1.047
	P					3.6E-01	4.6E-01
	s.e.					0.019	0.066
r^2/pseudo-r^2		0.000	0.007	0.000	0.007	0.000	0.007
n		92,122	79,889	92,122	79,889	92,122	79,889

Supplementary Table 5. Fully-adjusted model in women aged 60 and over at recruitment.

Risk factor included in model	Statistic	Terms included in addition to variables included in the fully adjusted model							
		Fully adjusted model		Adopted		Age at menarche		Birthweight	
		Cox	Logistic	Cox	Logistic	Cox	Logistic	Cox	Logistic
Amount smoked, pack-years (increasing)	HR/OR	1.004	1.011	1.004	1.011	1.004	1.010	1.005	1.014
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	8.7E-15	1.7E-14	1.0E-13
	s.e.	0.000	0.001	0.000	0.001	0.000	0.001	0.001	0.002
Alcohol intake frequency, daily or almost daily	HR/OR	0.951	0.776	0.950	0.774	0.950	0.779	0.934	0.843
	P	4.8E-03	7.9E-06	4.3E-03	6.5E-06	4.5E-03	1.5E-05	4.1E-03	2.6E-02
	s.e.	0.017	0.044	0.017	0.044	0.017	0.045	0.022	0.065
Alcohol intake frequency, three or four times a week	HR/OR	0.949	0.790	0.949	0.789	0.950	0.787	0.935	0.779
	P	2.6E-03	2.3E-05	2.4E-03	2.1E-05	3.5E-03	2.4E-05	3.7E-03	1.1E-03
	s.e.	0.016	0.044	0.016	0.044	0.017	0.045	0.022	0.059
Alcohol intake frequency, once or twice a week	HR/OR	0.949	0.809	0.947	0.807	0.946	0.805	0.933	0.809
	P	1.6E-03	5.7E-05	1.2E-03	4.4E-05	9.2E-04	4.7E-05	1.8E-03	3.3E-03
	s.e.	0.016	0.043	0.016	0.042	0.016	0.043	0.021	0.058
Alcohol intake frequency, one to three times a month	HR/OR	0.955	0.821	0.955	0.822	0.948	0.823	0.938	0.860
	P	1.5E-02	1.1E-03	1.4E-02	1.1E-03	5.4E-03	1.5E-03	1.1E-02	6.5E-02
	s.e.	0.018	0.050	0.018	0.050	0.018	0.050	0.024	0.070
Alcohol intake frequency, special occasions only	HR/OR	0.992	0.901	0.992	0.902	0.994	0.906	0.968	0.889
	P	6.5E-01	6.0E-02	6.6E-01	6.2E-02	7.4E-01	7.7E-02	1.8E-01	1.2E-01
	s.e.	0.018	0.050	0.018	0.050	0.018	0.051	0.023	0.068
Number of live births (increasing)	HR/OR	0.965	0.934	0.965	0.935	0.965	0.935	0.962	0.926
	P	<1.0E-15	1.5E-07	<1.0E-15	2.1E-07	<1.0E-15	3.0E-07	1.1E-13	1.2E-05
	s.e.	0.004	0.012	0.004	0.012	0.004	0.012	0.005	0.016
Highest educational level achieved (increasing)	HR/OR	0.991	0.935	0.991	0.935	0.990	0.933	0.991	0.936
	P	2.1E-04	<1.0E-15	1.5E-04	<1.0E-15	3.2E-05	<1.0E-15	3.3E-03	8.2E-10
	s.e.	0.002	0.007	0.002	0.007	0.002	0.007	0.003	0.010
Does not eat poultry, beef, lamb/mutton or pork	HR/OR	1.073	1.107	1.074	1.109	1.075	1.120	1.077	1.131
	P	2.5E-03	1.9E-01	2.4E-03	1.9E-01	2.1E-03	1.5E-01	1.5E-02	2.3E-01
	s.e.	0.025	0.087	0.025	0.087	0.025	0.088	0.033	0.117
Townsend deprivation index at recruitment (increasing deprivation)	HR/OR	1.004	1.015	1.004	1.015	1.005	1.017	1.006	1.015
	P	2.1E-02	3.6E-03	1.9E-02	3.3E-03	5.7E-03	1.7E-03	3.4E-03	3.2E-02
	s.e.	0.002	0.005	0.002	0.005	0.002	0.005	0.002	0.007
Body mass index (BMI) (increasing)	HR/OR	0.989	1.005	0.989	1.005	0.988	1.004	0.988	1.006
	P	<1.0E-15	1.3E-01	<1.0E-15	1.4E-01	<1.0E-15	2.3E-01	<1.0E-15	1.3E-01
	s.e.	0.001	0.003	0.001	0.003	0.001	0.003	0.001	0.004
Smoking status, previous	HR/OR	1.020	1.159	1.019	1.161	1.018	1.170	1.000	1.098
	P	1.7E-01	8.4E-04	1.7E-01	7.5E-04	2.0E-01	4.6E-04	9.9E-01	1.2E-01
	s.e.	0.014	0.051	0.014	0.051	0.015	0.052	0.019	0.067
Smoking status, current	HR/OR	1.095	1.388	1.097	1.390	1.103	1.403	1.084	1.282
	P	2.1E-04	1.9E-06	1.6E-04	1.6E-06	8.5E-05	1.2E-06	1.8E-02	1.0E-02
	s.e.	0.027	0.095	0.027	0.096	0.027	0.098	0.037	0.124
Adopted as a child, yes	HR/OR			0.933	1.104				
	P			7.2E-02	4.0E-01				
	s.e.			0.036	0.130				
Age of menarche (decreasing)	HR/OR					1.009	1.016		
	P					1.8E-03	1.1E-01		
	s.e.					0.003	0.010		
Birth weight (kg) (decreasing)	HR/OR							1.020	1.147
	P							3.0E-02	3.0E-06
	s.e.							0.009	0.034
r^2/pseudo-r^2		0.001	0.015	0.001	0.015	0.001	0.015	0.001	0.017
n		76,727	66,589	76,641	66,513	75,009	65,063	43,654	37,743

Risk factor included in model	Statistic	Breast fed		Comparative body size as child		Comparative height as child		Handedness	
		Cox	Logistic	Cox	Logistic	Cox	Logistic	Cox	Logistic
Amount smoked, pack-years (increasing)	HR/OR	1.005	1.012	1.004	1.011	1.005	1.011	1.004	1.011
	P	<1.0E-15	1.4E-14	<1.0E-15	<1.0E-15	<1.0E-15	1.3E-15	<1.0E-15	<1.0E-15
	s.e.	0.001	0.002	0.000	0.001	0.000	0.001	0.000	0.001
Alcohol intake frequency, daily or almost daily	HR/OR	0.959	0.791	0.947	0.781	0.950	0.788	0.951	0.775
	P	4.0E-02	3.6E-04	2.3E-03	1.5E-05	4.0E-03	3.4E-05	4.6E-03	7.6E-06
	s.e.	0.020	0.052	0.017	0.045	0.017	0.045	0.017	0.044
Alcohol intake frequency, three or four times a week	HR/OR	0.962	0.785	0.945	0.795	0.947	0.801	0.949	0.790
	P	5.6E-02	1.8E-04	1.2E-03	4.7E-05	1.9E-03	8.4E-05	2.5E-03	2.5E-05
	s.e.	0.019	0.051	0.017	0.045	0.017	0.045	0.016	0.044
Alcohol intake frequency, once or twice a week	HR/OR	0.959	0.797	0.945	0.811	0.948	0.825	0.949	0.810
	P	2.9E-02	2.2E-04	6.7E-04	7.8E-05	1.5E-03	3.1E-04	1.5E-03	5.8E-05
	s.e.	0.018	0.049	0.016	0.043	0.016	0.044	0.016	0.043
Alcohol intake frequency, one to three times a month	HR/OR	0.964	0.858	0.950	0.825	0.955	0.827	0.955	0.822
	P	9.6E-02	2.8E-02	6.6E-03	1.6E-03	1.5E-02	1.9E-03	1.5E-02	1.1E-03
	s.e.	0.021	0.060	0.018	0.050	0.018	0.051	0.018	0.050
Alcohol intake frequency, special occasions only	HR/OR	1.006	0.902	0.987	0.904	0.990	0.921	0.992	0.901
	P	7.7E-01	1.1E-01	4.7E-01	6.9E-02	5.8E-01	1.4E-01	6.5E-01	5.9E-02
	s.e.	0.021	0.058	0.018	0.050	0.018	0.052	0.018	0.050
Number of live births (increasing)	HR/OR	0.962	0.933	0.965	0.935	0.965	0.931	0.965	0.934
	P	<1.0E-15	3.0E-06	<1.0E-15	2.5E-07	<1.0E-15	4.9E-08	<1.0E-15	1.5E-07
	s.e.	0.004	0.014	0.004	0.012	0.004	0.012	0.004	0.012
Highest educational level achieved (increasing)	HR/OR	0.990	0.929	0.991	0.937	0.992	0.934	0.991	0.935
	P	3.6E-04	1.1E-15	3.1E-04	<1.0E-15	4.8E-04	<1.0E-15	2.3E-04	<1.0E-15
	s.e.	0.003	0.009	0.002	0.007	0.002	0.007	0.002	0.007
Does not eat poultry, beef, lamb/mutton or pork	HR/OR	1.081	1.195	1.073	1.133	1.074	1.111	1.074	1.106
	P	3.1E-03	4.1E-02	2.7E-03	1.1E-01	2.6E-03	1.8E-01	2.4E-03	2.0E-01
	s.e.	0.029	0.104	0.025	0.089	0.025	0.088	0.025	0.087
Townsend deprivation index at recruitment (increasing deprivation)	HR/OR	1.006	1.012	1.004	1.014	1.004	1.013	1.004	1.015
	P	2.7E-03	3.9E-02	3.1E-02	8.2E-03	2.4E-02	1.2E-02	2.0E-02	3.7E-03
	s.e.	0.002	0.006	0.002	0.005	0.002	0.005	0.002	0.005
Body mass index (BMI) (increasing)	HR/OR	0.989	1.005	0.989	1.006	0.988	1.004	0.989	1.005
	P	<1.0E-15	1.2E-01	<1.0E-15	7.7E-02	<1.0E-15	1.9E-01	<1.0E-15	1.4E-01
	s.e.	0.001	0.004	0.001	0.003	0.001	0.003	0.001	0.003
Smoking status, previous	HR/OR	1.007	1.131	1.018	1.157	1.019	1.165	1.020	1.161
	P	6.6E-01	1.6E-02	2.1E-01	1.0E-03	1.8E-01	6.2E-04	1.7E-01	7.7E-04
	s.e.	0.016	0.058	0.014	0.052	0.015	0.052	0.014	0.051
Smoking status, current	HR/OR	1.077	1.307	1.098	1.412	1.094	1.406	1.095	1.388
	P	9.2E-03	9.2E-04	1.7E-04	6.2E-07	2.8E-04	9.0E-07	2.1E-04	1.9E-06
	s.e.	0.031	0.105	0.027	0.098	0.027	0.098	0.027	0.095
Breastfed as a baby, no	HR/OR	0.994	1.066						
	P	6.0E-01	1.0E-01						
	s.e.	0.012	0.042						
Comparative body size at age 10, plumper	HR/OR			0.977	1.044				
	P			7.8E-02	3.1E-01				
	s.e.			0.013	0.045				
Comparative body size at age 10, thinner	HR/OR			0.998	1.160				
	P			8.5E-01	7.6E-06				
	s.e.			0.010	0.039				
Comparative height size at age 10, shorter	HR/OR					0.979	1.040		
	P					6.9E-02	3.0E-01		
	s.e.					0.011	0.039		
Comparative height size at age 10, taller	HR/OR					1.004	1.059		
	P					7.5E-01	1.1E-01		
	s.e.					0.011	0.038		
Handedness, both equally	HR/OR							0.967	1.115
	P							4.1E-01	3.8E-01
	s.e.							0.039	0.139
Handedness, left handed	HR/OR							0.994	0.889
	P							7.1E-01	4.0E-02
	s.e.							0.016	0.051
r^2/pseudo-r^2		0.001	0.015	0.001	0.015	0.001	0.015	0.001	0.015
n		58,260	50,639	75,664	65,666	75,467	65,494	76,717	66,581

Risk factor included in model	Statistic	Maternal smoking		Multiple birth		Year of birth		Allergy <20 year	
		Cox	Logistic	Cox	Logistic	Cox	Logistic	Cox	Logistic
Amount smoked, pack-years (increasing)	HR/OR	1.005	1.011	1.005	1.011	1.004	1.010	1.004	1.011
	P	<1.0E-15	1.2E-14	<1.0E-15	2.0E-15	<1.0E-15	2.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.001	0.001	0.000	0.001	0.000	0.001	0.000	0.001
Alcohol intake frequency, daily or almost daily	HR/OR	0.946	0.776	0.949	0.777	0.953	0.779	0.951	0.776
	P	3.9E-03	3.4E-05	3.2E-03	1.1E-05	6.9E-03	1.1E-05	4.8E-03	7.9E-06
	s.e.	0.018	0.047	0.017	0.045	0.017	0.044	0.017	0.044
Alcohol intake frequency, three or four times a week	HR/OR	0.949	0.794	0.947	0.788	0.954	0.801	0.949	0.790
	P	4.6E-03	1.2E-04	1.7E-03	2.4E-05	7.0E-03	6.8E-05	2.7E-03	2.3E-05
	s.e.	0.018	0.048	0.017	0.044	0.017	0.045	0.016	0.044
Alcohol intake frequency, once or twice a week	HR/OR	0.957	0.806	0.947	0.804	0.953	0.817	0.949	0.809
	P	1.4E-02	1.3E-04	1.3E-03	4.1E-05	3.4E-03	1.2E-04	1.6E-03	5.7E-05
	s.e.	0.017	0.046	0.016	0.043	0.016	0.043	0.016	0.043
Alcohol intake frequency, one to three times a month	HR/OR	0.961	0.843	0.953	0.822	0.958	0.829	0.955	0.821
	P	4.8E-02	8.2E-03	1.2E-02	1.3E-03	2.4E-02	1.9E-03	1.5E-02	1.1E-03
	s.e.	0.019	0.054	0.018	0.050	0.018	0.050	0.018	0.050
Alcohol intake frequency, special occasions only	HR/OR	0.998	0.918	0.990	0.902	0.993	0.904	0.992	0.901
	P	9.0E-01	1.5E-01	5.6E-01	6.4E-02	7.0E-01	6.8E-02	6.6E-01	6.0E-02
	s.e.	0.019	0.054	0.018	0.050	0.018	0.050	0.018	0.050
Number of live births (increasing)	HR/OR	0.964	0.921	0.965	0.935	0.964	0.930	0.965	0.934
	P	<1.0E-15	5.0E-09	<1.0E-15	2.8E-07	<1.0E-15	2.3E-08	<1.0E-15	1.5E-07
	s.e.	0.004	0.013	0.004	0.012	0.004	0.012	0.004	0.012
Highest educational level achieved (increasing)	HR/OR	0.992	0.931	0.991	0.935	0.993	0.939	0.991	0.935
	P	2.1E-03	<1.0E-15	2.4E-04	<1.0E-15	2.1E-03	2.4E-15	2.7E-04	<1.0E-15
	s.e.	0.003	0.008	0.002	0.007	0.002	0.007	0.002	0.007
Does not eat poultry, beef, lamb/mutton or pork	HR/OR	1.071	1.180	1.068	1.116	1.079	1.120	1.073	1.107
	P	6.5E-03	4.5E-02	4.9E-03	1.6E-01	1.2E-03	1.5E-01	2.4E-03	1.9E-01
	s.e.	0.027	0.097	0.025	0.088	0.025	0.088	0.025	0.087
Townsend deprivation index at recruitment (increasing deprivation)	HR/OR	1.005	1.013	1.004	1.015	1.004	1.015	1.004	1.015
	P	6.7E-03	2.1E-02	1.8E-02	3.9E-03	2.1E-02	3.2E-03	2.1E-02	3.6E-03
	s.e.	0.002	0.006	0.002	0.005	0.002	0.005	0.002	0.005
Body mass index (BMI) (increasing)	HR/OR	0.988	1.004	0.989	1.005	0.989	1.005	0.989	1.005
	P	<1.0E-15	2.5E-01	<1.0E-15	1.2E-01	<1.0E-15	1.1E-01	<1.0E-15	1.3E-01
	s.e.	0.001	0.003	0.001	0.003	0.001	0.003	0.001	0.003
Smoking status, previous	HR/OR	1.014	1.133	1.016	1.154	1.022	1.167	1.020	1.159
	P	3.6E-01	9.7E-03	2.7E-01	1.3E-03	1.3E-01	5.0E-04	1.7E-01	8.6E-04
	s.e.	0.016	0.055	0.015	0.052	0.014	0.052	0.014	0.051
Smoking status, current	HR/OR	1.092	1.333	1.086	1.386	1.101	1.409	1.095	1.387
	P	1.2E-03	1.6E-04	9.0E-04	2.7E-06	8.8E-05	6.1E-07	2.1E-04	2.0E-06
	s.e.	0.030	0.101	0.027	0.097	0.027	0.097	0.027	0.095
Maternal smoking around birth, yes	HR/OR	0.995	1.125						
	P	6.7E-01	7.3E-04						
	s.e.	0.011	0.039						
Part of a multiple birth, yes	HR/OR			1.093	1.540				
	P			2.7E-03	3.0E-07				
	s.e.			0.032	0.130				
Year of birth (decreasing)	HR/OR					1.007	1.022		
	P					4.1E-07	8.0E-06		
	s.e.					0.001	0.005		
Allergy <20 years	HR/OR							0.976	0.938
	P							2.6E-01	3.8E-01
	s.e.							0.021	0.069
r^2/pseudo-r^2		0.001	0.015	0.001	0.015	0.001	0.015	0.001	0.015
n		65,938	57,269	75,555	65,633	76,727	66,589	76,727	66,589

Risk factor included in model	Statistic	Cancer <20 years		Gynecological problems <20 years		Headache <20 years		Infection <20 years	
		Cox	Logistic	Cox	Logistic	Cox	Logistic	Cox	Logistic
Amount smoked, pack-years (increasing)	HR/OR	1.004	1.011	1.004	1.011	1.004	1.011	1.004	1.011
	P	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15	<1.0E-15
	s.e.	0.000	0.001	0.000	0.001	0.000	0.001	0.000	0.001
Alcohol intake frequency, daily or almost daily	HR/OR	0.951	0.776	0.951	0.776	0.950	0.773	0.951	0.776
	P	4.9E-03	8.2E-06	4.9E-03	8.0E-06	3.8E-03	6.2E-06	4.8E-03	8.0E-06
	s.e.	0.017	0.044	0.017	0.044	0.017	0.044	0.017	0.044
Alcohol intake frequency, three or four times a week	HR/OR	0.949	0.790	0.949	0.790	0.948	0.787	0.949	0.790
	P	2.7E-03	2.5E-05	2.6E-03	2.3E-05	2.1E-03	1.8E-05	2.6E-03	2.3E-05
	s.e.	0.016	0.044	0.016	0.044	0.016	0.044	0.016	0.044
Alcohol intake frequency, once or twice a week	HR/OR	0.949	0.810	0.949	0.809	0.948	0.807	0.949	0.809
	P	1.6E-03	5.9E-05	1.6E-03	5.6E-05	1.2E-03	4.5E-05	1.6E-03	5.7E-05
	s.e.	0.016	0.043	0.016	0.043	0.016	0.042	0.016	0.043
Alcohol intake frequency, one to three times a month	HR/OR	0.955	0.822	0.955	0.821	0.955	0.820	0.955	0.821
	P	1.6E-02	1.1E-03	1.5E-02	1.1E-03	1.4E-02	9.9E-04	1.5E-02	1.1E-03
	s.e.	0.018	0.050	0.018	0.050	0.018	0.049	0.018	0.050
Alcohol intake frequency, special occasions only	HR/OR	0.992	0.902	0.992	0.901	0.992	0.900	0.992	0.901
	P	6.6E-01	6.1E-02	6.6E-01	6.0E-02	6.3E-01	5.6E-02	6.6E-01	6.0E-02
	s.e.	0.018	0.050	0.018	0.050	0.018	0.050	0.018	0.050
Number of live births (increasing)	HR/OR	0.965	0.934	0.965	0.934	0.965	0.934	0.965	0.935
	P	<1.0E-15	1.5E-07	<1.0E-15	1.5E-07	<1.0E-15	1.5E-07	<1.0E-15	1.6E-07
	s.e.	0.004	0.012	0.004	0.012	0.004	0.012	0.004	0.012
Highest educational level achieved (increasing)	HR/OR	0.991	0.935	0.991	0.935	0.991	0.935	0.991	0.935
	P	2.1E-04	<1.0E-15	2.2E-04	<1.0E-15	2.5E-04	<1.0E-15	2.3E-04	<1.0E-15
	s.e.	0.002	0.007	0.002	0.007	0.002	0.007	0.002	0.007
Does not eat poultry, beef, lamb/mutton or pork	HR/OR	1.073	1.107	1.074	1.108	1.074	1.108	1.073	1.106
	P	2.5E-03	1.9E-01	2.3E-03	1.9E-01	2.4E-03	1.9E-01	2.5E-03	2.0E-01
	s.e.	0.025	0.087	0.025	0.087	0.025	0.087	0.025	0.087
Townsend deprivation index at recruitment (increasing deprivation)	HR/OR	1.004	1.015	1.004	1.015	1.004	1.015	1.004	1.015
	P	2.1E-02	3.6E-03	2.1E-02	3.6E-03	2.3E-02	3.8E-03	2.1E-02	3.8E-03
	s.e.	0.002	0.005	0.002	0.005	0.002	0.005	0.002	0.005
Body mass index (BMI) (increasing)	HR/OR	0.989	1.005	0.989	1.005	0.989	1.005	0.989	1.005
	P	<1.0E-15	1.3E-01	<1.0E-15	1.3E-01	<1.0E-15	1.3E-01	<1.0E-15	1.3E-01
	s.e.	0.001	0.003	0.001	0.003	0.001	0.003	0.001	0.003
Smoking status, previous	HR/OR	1.020	1.160	1.020	1.160	1.020	1.160	1.020	1.159
	P	1.7E-01	8.3E-04	1.7E-01	8.3E-04	1.6E-01	8.1E-04	1.7E-01	8.3E-04
	s.e.	0.014	0.051	0.014	0.051	0.014	0.051	0.014	0.051
Smoking status, current	HR/OR	1.095	1.388	1.096	1.388	1.095	1.386	1.095	1.388
	P	2.1E-04	1.9E-06	2.0E-04	1.8E-06	2.2E-04	2.0E-06	2.1E-04	1.8E-06
	s.e.	0.027	0.095	0.027	0.095	0.027	0.095	0.027	0.095
Cancer <20 years	HR/OR	1.065	1.748						
	P	7.7E-01	3.0E-01						
	s.e.	0.233	0.942						
Gynecological problems <20 years	HR/OR			0.893	0.726				
	P			3.1E-01	4.1E-01				
	s.e.			0.099	0.284				
Headache <20 years	HR/OR					0.919	0.795		
	P					2.6E-02	9.5E-02		
	s.e.					0.035	0.110		
Infection <20 years	HR/OR							0.986	1.086
	P							5.0E-01	2.3E-01
	s.e.							0.021	0.074
r^2/pseudo-r^2		0.001	0.015	0.001	0.015	0.001	0.015	0.001	0.015
n		76,727	66,589	76,727	66,589	76,727	66,589	76,727	66,589

Supplementary Table 6. Associations of the early-life risk factors birth weight, maternal smoking and part of a multiple birth with early menopause when included in the same model.

Model including birth weight, multiple birth, and maternal smoking	Partially adjusted			Fully adjusted		
	OR	s.e.	*P*	OR	s.e.	*P*
All ages						
Birth weight (kg) (decreasing)	1.12	0.024	9.1E-08	1.101	0.025	2.1E-05
Maternal smoking around birth, yes	1.141	0.032	3.3E-06	1.094	0.034	3.5E-03
Part of a multiple birth, yes	1.431	0.099	2.1E-07	1.458	0.108	3.5E-07
Aged 60 years and over						
Birth weight (kg) (decreasing)	1.103	0.033	9.9E-04	1.087	0.035	9.3E-03
Maternal smoking around birth, yes	1.203	0.051	1.5E-05	1.178	0.054	4.0E-04
Part of a multiple birth, yes	1.62	0.156	5.8E-07	1.663	0.173	1.0E-06

OR=odds ratio; s.e.=standard error.

Supplementary Table 7. Associations of the early-life risk factors age at menarche and comparative body size with early menopause when included in the same model.

		Partially adjusted			Fully adjusted		
Variable	Covariates in model	OR	s.e.	*P*	OR	s.e.	*P*
All ages							
Age at menarche	Body size at age 10	1.031	0.007	2.7E-06	1.04	0.007	3.0E-08
Comparative body size at age 10, plumper	Age at menarche	0.976	0.027	3.9E-01	0.967	0.029	2.8E-01
Comparative body size at age 10, thinner	Age at menarche	1.149	0.026	5.5E-10	1.126	0.027	1.2E-06
Aged 60 years and over							
Age at menarche	Year of birth, body size at age 10	1.014	0.009	1.4E-01	1.023	0.010	2.0E-02
Comparative body size at age 10, plumper	Year of birth, age at menarche	1.068	0.042	9.8E-02	1.046	0.045	3.0E-01
Comparative body size at age 10, thinner	Year of birth, age at menarche	1.169	0.036	4.4E-07	1.157	0.039	1.7E-05

OR=odds ratio; s.e.=standard error.

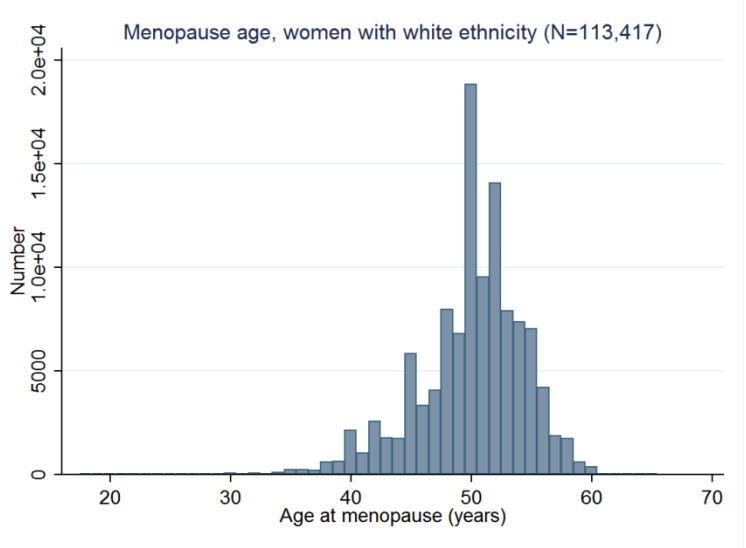
Supplementary Figure 1. Distribution of age at recruitment in early menopause cases and controls.

(i) All cohort.

(ii) Aged 60 and over at recruitment.

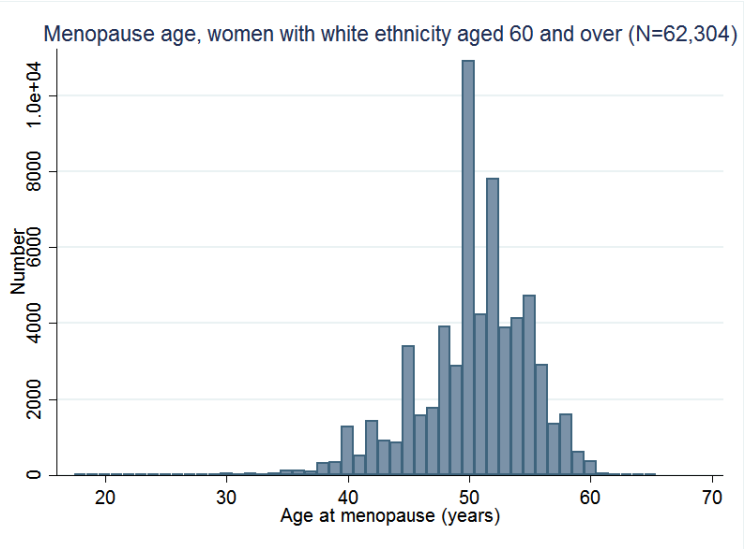
Supplementary Figure 2. Distribution of age at menopause in all women and women aged 60 and over at recruitment.

(i) All cohort.



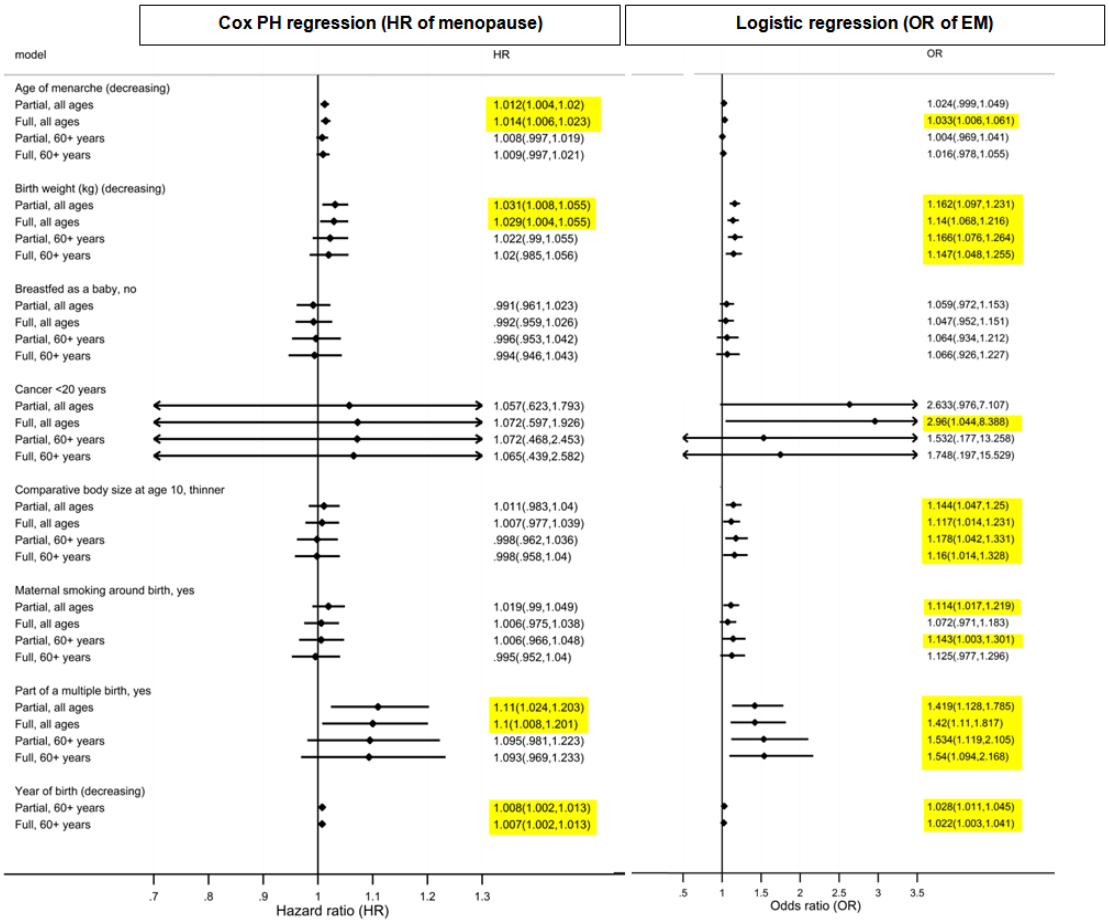
Median=50 years. Interquartile range (48,53). Mean=50.0 years. Range (18,65).

(ii) Aged 60 and over at recruitment.



Median=51 years. Interquartile range (48,54). Mean=50.4 years. Range (18,65).

Supplementary Figure 3. Associations between early-life risk factors and menopause.



HR=hazard ratio; OR=odds ratio.

Notes: Results shown are the hazard ratio of menopause in the Cox proportional hazards model or odds of early menopause in the logistic regression model. Results are for the partially- or fully- adjusted model including one early-life risk factor at a time. Confidence intervals are 99.995%. Highlighted results are significant at P<5×10-5.

Chapter 3:
Genome-wide association study with 1000 genomes imputation identifies signals for nine sex-hormone-related phenotypes

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Main text

Abstract

Genetic factors contribute strongly to sex-hormone levels, yet knowledge of the regulatory mechanisms remains incomplete. Genome-wide association studies (GWAS) have identified only a small number of loci associated with sex hormone levels, with several reproductive hormones yet to be assessed. The aim of the study was to identify novel genetic variants contributing to the regulation of sex hormones. We performed GWAS using genotypes imputed from the 1000 Genomes reference panel. The study used genotype and phenotype data from a UK twin register. We included 2,913 individuals (up to 294 males) from the Twins UK study, excluding individuals receiving hormone treatment. Phenotypes were standardized for age, sex, BMI, stage of menstrual cycle, and menopausal status. We tested 7,879,351 autosomal SNPs for association with levels of dehydroepiandrosterone sulphate (DHEAS), oestradiol, free androgen index (FAI), follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, progesterone, sex-hormone binding globulin and testosterone. Eight independent genetic variants reached genome-wide significance ($p<5\times10^{-8}$), with minor allele frequencies of 1.3%–23.9%. Novel signals included variants for progesterone ($p=7.68\times10^{-12}$), oestradiol ($p=1.63\times10^{-8}$) and FAI ($p=1.50\times10^{-8}$). A genetic variant near the *FSHB* gene was identified which influenced both FSH ($p=1.74\times10^{-8}$) and LH ($p=3.94\times10^{-9}$) levels. A separate locus on chromosome 7 was associated with both DHEAS ($p=1.82\times10^{-14}$) and progesterone ($p=6.09\times10^{-14}$). This study highlights loci that are relevant to reproductive function and suggests overlap in the genetic basis of hormone regulation.

Introduction

Studies have suggested that genetic factors contribute significantly to population variance in sex hormone levels, however few associated genetic variants and genes have been identified to date [1]. As well as playing an important role in reproduction, variations in sex hormone levels can have wider implications for health and disease. Reproductive functions include control of the menstrual cycle, spermatogenesis, steroidogenesis and lactation, and sex hormone levels have been implicated in breast cancer, cardiovascular disease, osteoporosis, type 2 diabetes and ageing [2-5]. Circulating levels of sex hormones are limited by sex-hormone binding globulin (SHBG), which is a glycoprotein that binds and transports oestradiol, testosterone and dehydroepiandrosterone (DHEA) to a lesser extent [6].

GWAS have been performed for dehydroepiandrosterone sulphate (DHEAS), SHBG, follicle-stimulating hormone (FSH), luteinizing hormone (LH), oestradiol and testosterone [2,4,7-10]. The largest of these GWAS was in over 28,000 males and females and identified 12 loci associated with differences in SHBG levels, including four loci with sex-specific genetic effects and considerable allelic heterogeneity at the *SHBG* gene locus [7]. A recent study in 3,495 Chinese men has identified a novel locus associated with oestradiol and FSH levels, and a further novel locus for oestradiol [10]. A GWAS of total testosterone in males identified three loci, including two in the *SHBG* gene, that were also associated with SHBG levels [9]. In an analysis of males and females combined, eight loci associated with DHEAS were identified, of which several were associated with changes in gene expression levels in pathways linked to ageing [4]. GWAS studies of sex-hormone related phenotypes have explained less than 10% of variance in oestradiol and SHBG, and less than 5% of variance in testosterone, DHEAS, and FSH [4,7,9,10].

In this study, we performed a 1000 Genomes imputed GWAS to identify novel genetic variants in sex-hormone-related phenotypes where either GWAS has not yet been performed or has not been performed at 1000-Genomes-density variant coverage.

Methods

Study population

The study included up to 2,913 individuals of European ancestry from the Twins UK study with genotype and phenotype data[11]. Twins UK is a supported access resource with all data access requests overseen by the Twins UK Resource Executive Committee. All studies have ethical approval from the Guy's and St Thomas' Ethics Committee (for further information see http://www.twinsuk.ac.uk/data-access/). The Twins UK cohort is 51% monozygotic and 49% dizygotic [11]. Individuals included in the analysis were mostly females, however a small number of males (maximum of 294) were also included (Supplemental Table 1). Individuals who were pregnant or currently receiving hormone replacement therapy or oral contraceptive treatments were excluded from the analysis. Twins UK samples have been included in previous GWAS of DHEAS and SHBG [4,7].

Phenotypes

Plasma levels of DHEAS, FSH, LH, oestradiol, progesterone, prolactin, SHBG and testosterone were measured by commercial ElectroChemiLuminescent immunoassays on a Modular Analytics E170 analyser (Roche Diagnostics GmbH) using the prescribed assay calibrators and performed according to the manufacturer's protocol. The specific assays used were: DHEA-S (03000087; CalSet 03000095), FSH (11775863; CalSet II 03032680), LH (11732234; CalSet II 03561097), Estradiol II (03000079; CalSet II 03064921), Progesterone II (12145383; CalSet 12145391), Prolactin II (03203093; CalSet 03277356), SHBG (03052001: CalSet 03052028) and Testosterone II (05200067;CalSet II 05202230). Details of the immunoassays are provided in the Supplementary Information. Free androgen index (FAI) was calculated as (testosterone/sex hormone binding globulin) × 100 [12]. Individual sex hormone measures were fitted in a regression model against age, sex, BMI, phase of menstrual cycle (for females, as a categorical variable), menopausal status, after which the residuals were transformed to approximate a normal distribution (either through log, square-root or inverse rank normal transformation) and outliers more than four standard deviations from the mean were removed. Single-nucleotide polymorphism (SNP) beta estimate effect sizes are quoted as a per-allele

standard deviation change in the covariate-adjusted transformed residuals. The number of individuals included in the analysis of each hormone was 2,899 for DHEAS, 2,906 for oestradiol, 2,699 for FAI, 2,885 for FSH, 2,881 for LH, 2,865 for prolactin, 2,689 for progesterone, 2,913 for SHBG and 2,657 for testosterone (differences in the numbers for FAI and testosterone are accounted for by removal of outliers prior to inclusion in the GWAS).

Genotypes

Genotyping of the TwinsUK dataset was done with HumanHap300, HumanHap610Q, HumanHap1M Duo and HumanHap1.2M Duo 1M arrays. Imputation was done in two datasets (n=2,040 from the HumanHap300 array; n=3,614 from the HumanHap610Q, HumanHap1M Duo and 1.2M Duo 1M arrays) which were then merged with GTOOL. We performed imputation for Twins UK study subjects based on 1000 Genomes data as described previously [13]. This involved estimating the phase of contiguous variants in the subjects using the haplotypes calculated from the 1000 Genomes Project consisting of 1,094 individuals and 2,188 haplotypes and the program MACH 1.0.16. The variants in the build-37 November 2010 release of 1000 Genomes (Phase 1-α interim) were imputed into the phased haplotypes using MINIMAC. This resulted in 37,426,733 imputed SNPs. We excluded SNPs that were imputed with an r^2_{imp}<0.5. This left 10,879,115 SNPs and after filtering for MAF>0.01 the number fell to 7,879,351. We used a multi-ethnic reference panel that included 381 Europeans (including 98 Tuscans), 181 Americans, 246 Africans and 286 Asians to improve the quality of imputation, particularly at lower frequency variants [14].

Statistical analysis

We performed a linear mixed-model GWAS analysis for each of the hormones using the program GEMMA [15], which is capable of accounting for relatedness of the study subjects when applicable, as well as population stratification and cryptic relatedness. Association statistics using the score test were calculated for 7,879,351 autosomal SNPs passing a MAF filter of 0.01 and an imputation quality score of 0.5.

The Bonferroni corrected p-value for the number of SNPs tested across nine traits was $p<7\times10^{-10}$, however this is likely to be conservative given that many SNPs are unlikely to be independent and there are Bayesian arguments for less conservative p-values[13]. Hence, we considered independent significant SNPs to be those with $p<5\times10^{-8}$ and more than 1Mb away from another significant SNP. The UCSC Genome Browser and Locus Zoom were used to identify genes in the regions where significant SNPs were identified [16,17]. SNAP, HaploReg v2, Locus Zoom and Ensembl Biomart were used to identify HapMap proxies for the 1000 Genomes signals, with linkage disequilibrium evaluated in the 1000 Genomes Phase I CEU population[17-20]. We analysed expression quantitative trait loci (eQTL) data to identify associations between SNPs associated with variation in hormone levels and expression levels of nearby genes in the Multiple Tissue Human Expression Resource (MuTHER) [21]. Functional annotation of SNPs in strong linkage disequilibrium with the significant signals ($r^2>0.8$) was performed using wANNOVAR, GWAVA and HaploReg v2 [20,22,23].

Results

Hormone phenotypes are correlated

There were strong correlations between three groups of phenotypes included in our study (Table 1): (i) FAI, SHBG and testosterone; (ii) progesterone, DHEAS and testosterone; and, (iii) FSH and LH. FAI was positively correlated with testosterone (r=0.69) and negatively correlated with SHBG (r=-0.61), as would be expected since FAI is a calculated index of the amount of androgen not bound by SHBG. Testosterone and SHBG were not correlated (r=0.04). Progesterone was positively correlated with DHEAS (r=0.60) and, to a lesser extent, testosterone (r=0.44). As a result of the correlation with testosterone, progesterone was also correlated with FAI (r=0.39). DHEAS was also positively correlated with testosterone (r=0.55) and, as a result, FAI (r=0.52). There was a strong positive correlation between FSH and LH (r=0.63). Though the other correlations were smaller, oestradiol was positively correlated with testosterone (r=0.22) and was negatively correlated with FSH (r=-0.24).

Table 1. Correlation coefficients between the sex-hormone-related phenotypes.

		Oestradiol	Prolactin	DHEAS	SHBG	Progesterone	LH	FSH	Testosterone
		log_{10} (Oestradiol residuals)	log_{10} (Prolactin residuals)	square root (DHEAS residuals)	ln (SHBG residuals)	log_{10} (Progesterone residuals)	LH residuals	inverse normal (FSH residuals)	Testosterone residuals
Oestradiol	log_{10} (Oestradiol residuals)	1	-	-	-	-	-	-	-
Prolactin	log_{10} (Prolactin residuals)	**0.09**	1	-	-	-	-	-	-
DHEAS	square root (DHEAS residuals)	**0.09**	-0.01	1	-	-	-	-	-
SHBG	ln (SHBG residuals)	**0.08**	**0.06**	**-0.15**	1	-	-	-	-
Progesterone	log_{10} (Progesterone residuals)	**0.17**	**0.04**	**0.60**	**-0.06**	1	-	-	-
LH	LH residuals	0.00	**0.14**	0.03	0.03	0.04	1	-	-
FSH	inverse rank normal transformed (FSH residuals)	**-0.24**	**0.08**	0.01	-0.01	0.00	**0.63**	1	-
Testosterone	Testosterone residuals	**0.22**	0.03	**0.55**	**0.04**	**0.44**	**0.06**	-0.01	1
FAI	FAI residuals	**0.11**	0.00	**0.52**	**-0.61**	**0.39**	0.02	-0.01	**0.69**

Note: Values in bold are significant at $p<0.05$.

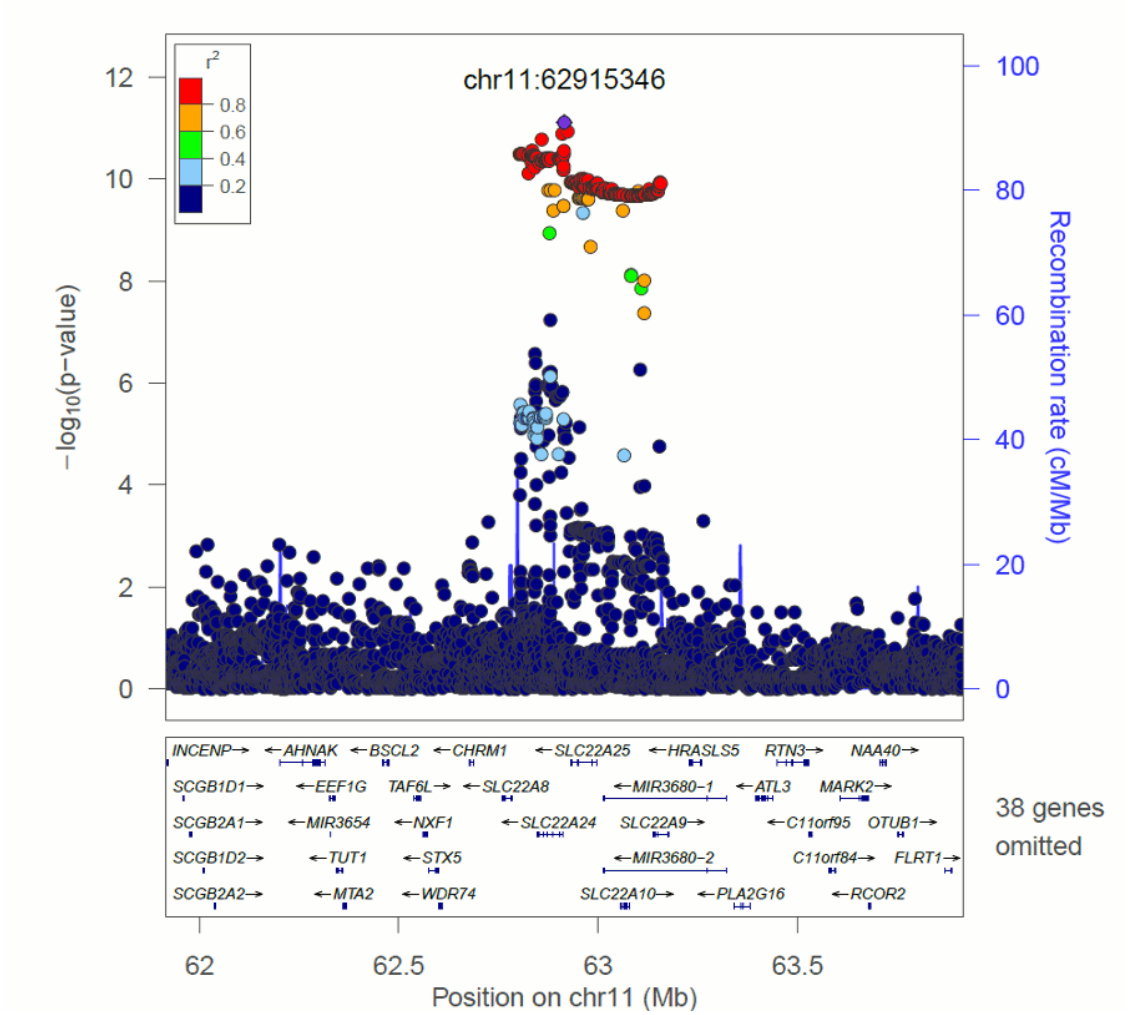
Three novel association signals

We identified new signals for progesterone, oestradiol and FAI (Table 2). The signal for progesterone (rs112295236, $p=7.68\times10^{-12}$, MAF=0.06) was identified in an intergenic region of chromosome 11 (Figure 1). We searched for associations of the progesterone SNP with other traits that might be influenced by progesterone levels, but we found no evidence for association with other phenotypes ($p>0.05$ in published GWAS of age at menopause, early menopause, age at menarche, BMI, height, type 2 diabetes and glycaemic traits, endometriosis, and birth weight (maternal and foetal genotype)) or in a GWAS of pre-term delivery (five gestational age/pre-term delivery traits for mother's and child's genotype, unpublished data). The signals for FAI and oestradiol were located at 16q12.2 (rs117145500, near *LOC643714*, $p=1.50\times10^{-8}$, MAF=0.06) and 12p13.31 (rs117585797, in *ANO2*, $p=1.63\times10^{-8}$, MAF=0.01) respectively, and demonstrated no association with other tested complex traits.

Five signals in known regions

We identified two signals that replicated previous associations, and a further three that have been reported previously, but for other phenotypes (Table 2). The signals for SHBG (rs1641549, near *SHBG* gene, $p=1.21\times10^{-15}$, MAF=0.24) and DHEAS (rs148982377, in *ZNF789*, $p=1.82\times10^{-14}$, MAF=0.04) have both been reported previously [4,7,9] (Supplemental Table 2). The significant associations for FSH (rs11031005; $p=1.74\times10^{-8}$, MAF=0.13) and LH (rs11031002; $p=3.94\times10^{-9}$, MAF=0.12) are highly correlated SNPs ($r^2 = 0.79$), residing in an intergenic region near the *FSHB* gene, and are in linkage disequilibrium with a published variant for menopause age (rs12294104) ($r^2=0.37$ for FSH (rs11031005) and $r^2=0.45$ for LH (rs11031002)) [24]. The strongest association for progesterone (rs34670419; $p=6.09\times10^{-14}$, MAF=0.04) was located on chromosome 7 in the 3' untranslated region of *ZKSCAN5* (Table 2; Supplemental Figure 1), a locus previously reported as associated with DHEAS levels.

Figure 1. SNPs within 1Mb of the significant signal for progesterone on chromosome 11 (rs112295236; chr11.hg19:g.62915346C>G).



Note: Not all genes are shown. Linkage disequilibrium is based on 1000 Genomes Nov 2010 EUR.

Table 2. Variants significantly associated with hormone levels ($p<5\times10^{-8}$).

Hormone[1]	Chr-position[2]	SNP id	Imputation quality	Minor allele frequency	Minor allele effect (% of s.d.)[3]	P-value	Location (gene)	Best candidate (distance)	Comments
DHEAS	chr7.hg19:g.99075038T>C	rs148982377T>C	0.927	0.038	-53.1	1.82×10^{-14}	*ZNF789* (intron)	*CYP3A7* (228kb)	Published signal for DHEAS[4]. Same signal as rs34670419 (progesterone)[5].
FAI	chr16.hg19:g.52947630A>C	rs117145500A>C	0.959	0.063	-35.9	1.50×10^{-8}	intergenic	*LOC643714* (307kb)	New signal.
FSH	chr11.hg19:g.30226356T>C	rs11031005T>C	0.978	0.129	-23.2	1.74×10^{-8}	intergenic	*FSHB* (26kb)	New signal. Overlaps with LH signal (rs11031002)[5].
LH	chr11.hg19:g.30215261T>A	rs11031002T>A	0.971	0.121	25.2	3.94×10^{-9}	intergenic	*FSHB* (37kb)	New signal. Overlaps with FSH signal (rs11031005)[5].
Oestradiol	chr12.hg19:g.60011490C>A	rs117585797C>A	0.572	0.013	87.1	1.63×10^{-8}	*ANO2* (intron)	*ANO2* (intronic)	New signal.
Progesterone	chr7.hg19:g.99130834G>T	rs34670419G>T	0.927	0.037	-55.6	6.09×10^{-14}	*ZKSCAN5* (3' UTR)	*CYP3A4* (224kb) or *CYP3A7* (172kb)	Published signal for DHEAS[4]. Same signal as rs148982377 (DHEAS)[5].
Progesterone	chr11.hg19:g.62915346C>G	rs112295236C>G	0.962	0.062	41.0	7.68×10^{-12}	intergenic	*SLC22A9* (222kb)	New signal.
SHBG	chr17.hg19:g.7574775C>T	rs1641549C>T	0.895	0.239	-28.0	1.21×10^{-15}	*TP53* (intron)	*SHBG* (38kb)	Published signal for testosterone[4].

Chr = chromosome; DHEAS = dehydroepiandrosterone sulphate; FAI = free androgen index; FSH = follicle-stimulating hormone; LH = luteinizing hormone; SHBG = sex-hormone binding globulin; s.d. = standard deviation; UTR = untranslated region. Notes: [1]Results are for square root of the DHEAS residuals and FAI residuals; inverse rank normal transformed FSH residuals and LH residuals; log10 of the oestradiol residuals and, progesterone residuals; and ln of the SHBG residuals; [2]Details of the reference sequence on which the variant descriptions are based is given in the Supplementary Information; [3]Minor allele effect sizes are quoted as a per-allele change expressed as a percentage of a standard deviation in the covariate-adjusted transformed residuals; [4]Further details regarding the published genetic variants associated with reproductive hormones are given in Supplemental Table 2; [5]Effect sizes and p-values for each signal in each hormone phenotype are given in Supplemental Table 3.

Two pairs of phenotypes have common signals

We identified overlaps between signals for FSH/LH and progesterone/DHEAS (association results for significant signals in all phenotypes are in Supplemental Table 3). The signals for FSH and LH were in linkage disequilibrium (r^2=0.79) and the most significant SNP for FSH also reached genome-wide significance for LH, however the direction of effects was the opposite of that expected by the phenotypic correlation (i.e. levels of FSH and LH are positively correlated, though the minor allele decreased FSH and increased LH (Supplemental Table 3). The strongest signal for progesterone on chromosome 7 (rs34670419) was in linkage disequilibrium with the signal for DHEAS (r^2=1), with allelic effects consistent with the expected phenotypic correlation (Supplemental Table 3).

Overlap between DHEAS and progesterone variants

To investigate the genetic overlap of DHEAS and progesterone further, we tested whether five published variants for DHEAS (identified prior to conditional analysis) were associated with progesterone levels in our data [4]. One of these variants reached genome-wide significance in our progesterone data (rs11761528, p=3.34 x10^{-8}) (Supplemental Table 4), and was at the same locus as our strongest progesterone signal (rs34670419, chr7:99130834). The published signal was not the strongest signal in our analysis though it was 12kb from and in moderate linkage disequilibrium with our top chromosome 7 progesterone signal (r^2=0.49). However, four of the five published variants were consistent in direction of effect (p=0.19) and two were nominally significant after Bonferroni correction (Supplemental Table 4). In addition we investigated whether our two progesterone signals were significant in other published DHEAS meta-analysis data by looking up our two progesterone signals in data from Zhai et al[4]. A proxy for our progesterone signal on chromosome 7 (r^2=0.58) was strongly associated with DHEAS (p=2.34x10^{-34}) and our progesterone signal on chromosome 11 showed weak evidence of association (p=1.53$\times10^{-4}$) (Supplemental Table 5) [4].

FSH and LH signals overlap with a menopause locus

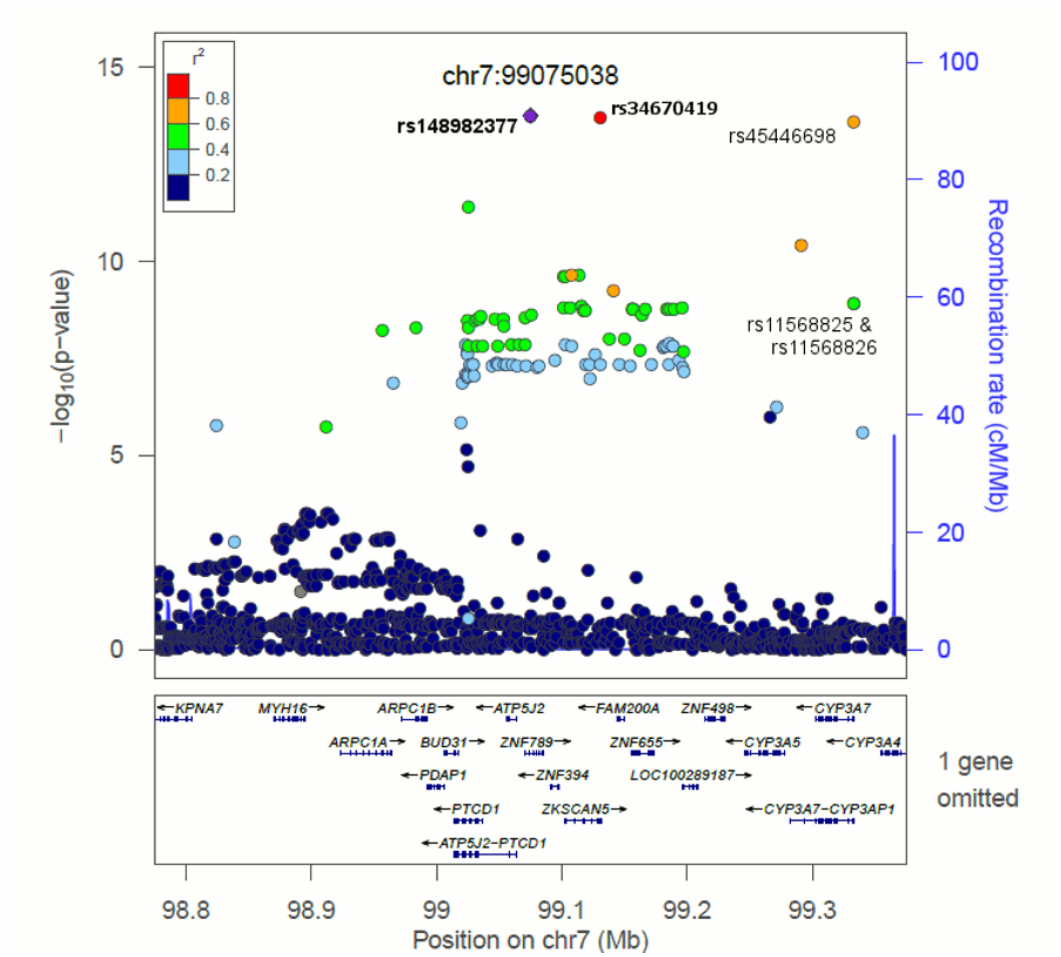
There was evidence of overlap between the signals for FSH and LH with a variant for menopause age on chromosome 11 (Supplemental Tables 6–9).

There was moderate linkage disequilibrium between the signals for FSH and LH and a published variant for menopause age (rs12294104), with r^2=0.37 for FSH (rs11031005) and r^2=0.45 for LH (rs11031002), p= 3.02×10^{-7} for FSH and p=6.25×10^{-7} for LH [24,25]. None of the other published menopause or menarche variants were associated with FSH or LH at Bonferroni corrected p<0.05 [24,26].

Identification of potentially causal candidate genes

The signal for SHBG (rs1641549) was 38 kb from the protein coding gene *SHBG*. Four other signals were in linkage disequilibrium with known polymorphisms that have functional consequences: The signals for DHEAS (rs148982377) and progesterone (rs34670419) were in linkage disequilibrium with three SNPs (rs45446698, rs11568825 and rs11568826; r^2>0.4 for all) that are part of a polymorphism in the promoter of *CYP3A7*, which is known to be associated with lower DHEAS levels (Figure 2). All three of these SNPs were genome-wide significant for DHEAS (p= 2.49×10^{-14} for rs45446698; p=1.18×10^{-9} for rs11568825 and rs11568826) and one was genome-wide significant for progesterone (rs45446698, p=$7.7\text{x}10^{-11}$), with the other two almost reaching significance (p<5×10^{-7}). The top signals for LH and FSH were within 38kb of, and in moderate linkage disequilibrium with, a known polymorphism (rs10835638) in the promoter of *FSHB* (rs11031005, r^2=0.62; rs11031002, r^2=0.74) (Supplemental Figure 2) [27]. Although the published *FSHB* promoter polymorphism was not the strongest signal in this region it was genome-wide significant for association with LH in our data (p=4.84×10^{-9}), and nearly significant for FSH (p=2.31×10^{-7}) (Supplemental Table 11). Other candidate genes were identified by a search of a 300kb region around each signal and are listed in Supplemental Table 10. Functional annotation of the signals with wANNOVAR did not reveal any additional likely causative variants.

Figure 2. SNPs within 300kb of the significant signal for DHEAS on chromosome 7 (rs148982377; chr7.hg19:g.99075038T>C).



Notes:

SNPs indicated are the strongest progesterone signal (rs34670419 (chr7.hg19:g.99130834G>T)) and those in the *CYP3A7* promoter polymorphism that were identified in this analysis (rs45446698 (chr7.hg19:g.99332948T>G), rs11568825 (chr7.hg19:g.99332986A>C), and rs11568826 (chr7.hg19:g.99332978A>T)).

Not all genes are shown. Linkage disequilibrium is based on 1000 Genomes Nov 2010 EUR.

Discussion

In this study of nine sex-hormone-related phenotypes, we identified three new signals and two pairs of phenotypes with a common signal. Four of the eight significant signals reached a conservative significance level of $p<7\times10^{-10}$, including the new signal for progesterone. This is the first published GWAS for the hormones progesterone, prolactin and the hormone measure FAI and we identified genetic associations for all except prolactin. As we are not aware of any other genotyped cohorts with measurements for progesterone and FAI, we have been unable to replicate these findings. The hormones DHEAS, FSH, LH, SHBG, testosterone and oestradiol have been included in previously published GWAS, and we have compared our results to these existing data [2,4,7-10]. This study is, to our knowledge, one of the first published GWAS of hormones using 1000 Genomes Phase I imputed data. Three of the signals we identified were low frequency (less than 5%) and had large effect sizes (more than 50% change relative to standard deviation). In addition to identifying novel signals, we also observed two previously identified signals, demonstrating that true signals can be identified for these traits even in modest sample sizes.

The progesterone signal (rs112295236) that we identified on chromosome 11 was upstream of *SLC22A9*, which codes for an organic anion transporter OAT7 found in the liver. OAT7 is involved with the transport of DHEAS and oestrone-3-sulphate in exchange for butyrate, and is thought to be important for the release of oestrogen-3-sulphate into the blood [28]. We did not find evidence to support this hypothesis in expression data, however such data are only currently available for a limited range of tissues (skin, lymphoblastoid cell lines and adipose), not including ovary, which is the main site of progesterone synthesis. However, there was evidence of an association between this progesterone signal and DHEAS levels in data from a published GWAS, albeit at sub-genome-wide significance levels [4], and DHEAS and progesterone were both strongly correlated in our study (r=0.60). We postulated that the progesterone variant may be associated with phenotypic outcomes, such as offspring birth weight, or age at menopause, but we found no associations in data from other studies. Of course the effect size of our variant was relatively small and we may be underpowered to detect additional phenotypic associations.

The variant associated with decreased FAI in our analyses (rs117145500), showed some evidence of association with increased SHBG and decreased DHEAS, consistent with the role of DHEA in testosterone synthesis and the effect of SHBG on the amount of free-androgens. This was supported by a negative correlation of FAI with SHBG in our data (r=-0.61) and positive correlation with DHEAS (r=0.55).

We identified a locus showing borderline significant association with oestradiol that requires further replication. The genetic variant associated with oestradiol (rs117585797) is a low frequency variant (minor allele frequency=0.013) with a large effect size, and is in an intron of the *ANO2* gene on chromosome 12. A previous GWAS of oestradiol levels in postmenopausal women did not identify any genetic variants reaching genome-wide significance in this region, though this may have been underpowered to detect this signal[2]. Two other genes are present within the same chromosomal region (*vWF* and *CD9*), though there is not strong evidence to support one as a more likely candidate over the other. *VWF* (47kb away) codes for the von Willebrand factor (vWF) protein which is involved in haemostasis, aiding platelet adhesion and preventing factor VIII degradation. Oestradiol has been shown to increase vWF production by endothelial cells *in vitro* [29], and in post-menopausal women, oral oestrogen treatment has been shown to increase vWF (though transdermal treatment did not show this effect) [30]. *CD9* (298kb away) is a widely expressed cell surface molecule that has been shown to be required for sperm–egg fusion in mice [31].

We provide evidence for overlap in the genetic regulation of two pairs of hormones whose levels are strongly correlated: FSH and LH (r=0.63); and progesterone and DHEAS (r=0.6). The FSH/LH variants were in linkage disequilibrium with a functional polymorphism (-211 G→T) in a progesterone response element of the promoter of the *FSHB* gene, which codes for the beta polypeptide of FSH. In females, FSH receptors are reported in endometrium [32] and in granulosa cells. In granulosa cells stimulation with FSH augments the expression of LH receptors [33]. *In vitro* studies have demonstrated that the allele in linkage disequilibrium with the effect alleles in our study reduces levels of FSHB expression [34,35]. Previous studies provide conflicting data regarding the direction of effect of this polymorphism on FSH and LH levels [27,36,37]. In our study, despite a positive overall correlation between FSH and LH levels, the

genetic variants were negatively associated with FSH and positively associated with LH. While this appears counter-intuitive, a similar situation is seen for other traits, e.g. genetic variants that increase fasting glucose are not always risk factors for type 2 diabetes [38]. Thus the relationship between FSH and LH is complex and will involve additional genetic and non-genetic factors. A previous GWAS including FSH and LH did not find this signal, though this study was in Chinese men[10] compared with our study of mainly female Europeans. Our study sheds light on part of the biological interaction between FSH and LH but further variants need to be identified to understand this more fully.

The second pair of hormones with overlap in genetic regulation was DHEAS and progesterone. We identified a signal for progesterone on chromosome 7 in linkage disequilibrium with a known signal for DHEAS, plus evidence for association of progesterone levels with 6/8 known DHEAS SNPs and of DHEAS levels with our newly identified chromosome 11 progesterone SNP. Both progesterone and DHEAS are steroid hormones that have a common precursor in their synthesis pathways (pregnenolone), but neither are directly synthesised from each other [3]. These hormones were positively correlated in our data (r=0.60). The top signal for DHEAS in our study was rs148982377, which tagged a polymorphism in the promoter of *CYP3A7*, which was also in linkage disequilibrium with the top progesterone signal. *CYP3A7* has a progesterone response element that is thought to regulate expression during pregnancy [39-41]. In the same region is the *CYP3A4* gene, which codes for a cytochrome P450 enzyme that metabolises progesterone, DHEAS, oestrone and testosterone [42].

The main limitation of our study is the absence of suitable replication cohorts for the hormone measures progesterone, oestradiol and FAI. Half of the signals reached a conservative, Bonferonni-adjusted significance level of $p<7\times10^{-10}$, giving us confidence in the findings for progesterone, DHEAS and SHBG. Although the signals for FAI, FSH, LH and oestradiol reached a less conservative significance threshold, there are strong arguments for the validity of less stringent p-values in 1000 Genomes imputed GWAS [13]. Further large studies are required to enable validation of our results. Such studies should also allow detection of new signals since the power of our study was limited by sample size and by the relatedness of the study individuals. Approximately 10% of our cohort consisted of males, and thus we will have been more likely to

detect genetic effects in females than males, though our ability to detect genetic variants affecting both sexes should not have been affected. The cohort used in our study was of European ancestry and as such our findings will need to be replicated in other ethnic populations. Further GWAS studies of these hormones may benefit from an increase in power by implementing newly emerging multi-variate methods which would take account of correlations between the hormones[43]. Any additional studies should also consider the need to directly quantify the effect of the variants, since it is difficult to relate the adjusted and transformed hormone measure that we used for our analysis to actual physiological changes. Further studies are required to address these issues.

In this GWAS of nine sex-hormone-related phenotypes, we were able to detect three new signals (oestradiol, FAI and progesterone traits), two pairs of signals overlapping with other traits (FSH/LH and progesterone/DHEAS) and two signals seen before (DHEAS and SHBG traits). We have demonstrated potential overlap in the genetics of hormone regulation, as might be expected from common pathways in hormone synthesis. As well as the overlap in the top signals for DHEAS and progesterone, and FSH and LH, there were other variants associated with more than one hormone at lower significance levels, suggesting further commonality in hormone regulation. We identified novel genetic variants and potential overlap in the genetic basis of hormone regulation that will inform future studies, not only of hormones but also of common diseases, ageing and reproductive lifespan that include sex related hormones on the aetiological pathway.

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Conflict of interest

The authors declare no conflict of interest.

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Supplementary Methods.

Details of the ElectroChemiLuminescent immunoassays

Roche Diagnostics Elecsys 170

Test Principle : Sandwich Electrochemiluminescence Immunoassay (ECLIA) using Streptavidin-coated Microparticles and with Ruthenium complex

SHBG

Measuring range 0.35 to 200 nmol/L
Limit of detection 0.35 nmol/L
Inter assay Precision
1.1% at a level of 15 nmol/L
1.3% at a level of 46 nmol/L
1.7% at a level of 219 nmol/L
Intra assay Precision
1.8% at a level of 14 nmol/L
2.1% at a level of 42 nmol/L
4.0% at a level of 189 nmol/L

Oestradiol

Measuring range 18 to 15780 pmol/L
Limit of detection 18 pmol/L
Interassay Precision
1.2% at a level of 130 nmol/L
1.7% at a level of 467 nmol/L
2.0% at a level of 4681 nmol/L
Intra assay Precision
4.7% at a level of 120 nmol/L
2.5% at a level of 472 nmol/L
2.2% at a level of 4693 nmol/L

Prolactin

Measuring range 1 to 10000 mU/L

Limit of detection 1 mU/L

Interassay Precision

0.8% at a level of 182 mU/L

1.7% at a level of 598 mU/L

1.1% at a level of 2314 mU/L

Intra assay Precision

1.8% at a level of 288 mU/L

1.4% at a level of 871 mU/L

1.6% at a level of 4477 mU/L

FSH

Measuring range 0.1to 200 U/L

Limit of detection 0.1 U/L

Interassay Precision

2.6% at a level of 6.0 U/L

2.8% at a level of 54 U/L

2.5% at a level of 178 U/L

Intra assay Precision

3.6% at a level of 5.3 U/L

3.7% at a level of 46 U/L

4.5% at a level of 229 U/L

LH

Measuring range 0.1 to 200 U/L

Limit of detection 0.1 U/L

Interassay Precision

1.2% at a level of 6.2 U/L

0.7% at a level of 92 U/L

0.9% at a level of 164 U/L

Intra assay Precision

2.0% at a level of 5.8 U/L

1.6% at a level of 89 U/L

2.2% at a level of 159 U/L

DHEA-S

Measuring range 0.003 to 27 umol/L

Limit of detection 0.003 umol/L

Interassay Precision

3.2% at a level of 2.6 umol/L

2.6% at a level of 10.9 umol/L

2.3% at a level of 21.3 umol/L

Intra assay Precision

2.5% at a level of 2.5 umol/L

2.7% at a level of 10.7 umol/L

2.4% at a level of 20.4 umol/L

Progesterone

Measuring range 0.1 to 191 nmol/L

Limit of detection 0.1 nmol/L

Interassay Precision

2.9% at a level of 2.3 nmol/L

1.4% at a level of 9.6 nmol/L

0.9% at a level of 103 nmol/L

Intra assay Precision

4.8% at a level of 2.5 nmol/L

2.8% at a level of 10.0 nmol/L

2.0% at a level of 112 nmol/L

Testosterone

Measuring range 0.09 to 52 nmol/L

Limit of detection 0.09 nmol/L

Interassay Precision

14.8% at a level of 0.32 nmol/L

4.1% at a level of 2.42 nmol/L

2.8% at a level of 7.4 nmol/L

2.1% at a level of 45.8 nmol/L

Intra assay Precision

18.1% at a level of 0.32 nmol/L

4.4% at a level of 2.42 nmol/L

3.2% at a level of 7.4 nmol/L

2.5% at a level of 45.8 nmol/L

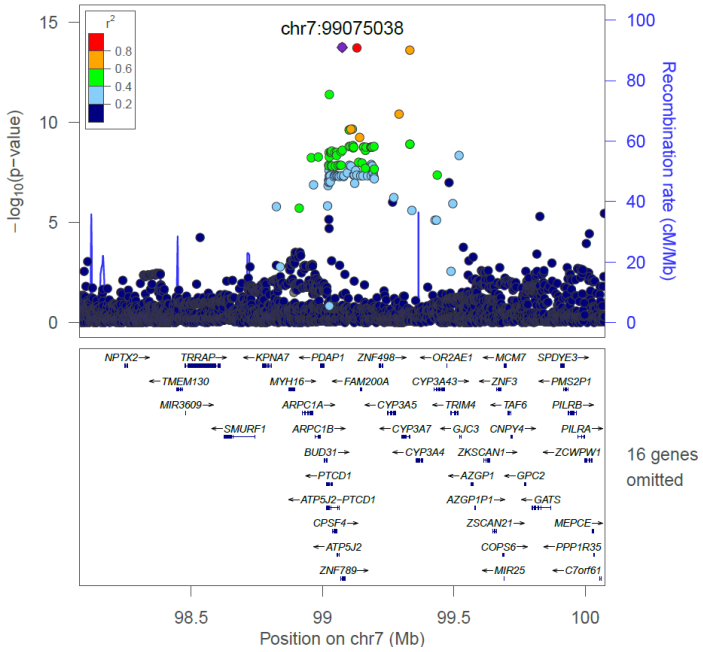
Supplementary Information on Variants

SNP id	Hormone	Chr-position	HGVS name
rs148982377T>C	DHEAS	chr7.hg19:g.99075038T>C	NT_007933.15:g. 99075038T>C
rs34670419G>T	Progesterone	chr7.hg19:g.99130834G>T	NT_007933.15:g.99130834G>T
rs11031002T>A	LH	chr11.hg19:g.30215261T>A	NT_009237.18:g.30215261T>A
rs11031005T>C	FSH	chr11.hg19:g.30226356T>C	NT_009237.18:g.30226356T>C
rs112295236C>G	Progesterone	chr11.hg19:g.62915346C>G	NT_167190.1:g.62915346C>G
rs117585797C>A	Oestradiol	chr12.hg19:g.6011490C>A	NT_009759.16:g.6011490C>A
rs117145500A>C	FAI	chr16.hg19:g.52947630A>C	NT_010498.15:g.52947630A>C
rs1641549C>T	SHBG	chr17.hg19:g.7574775C>T	NT_010718.16:g.7574775C>T

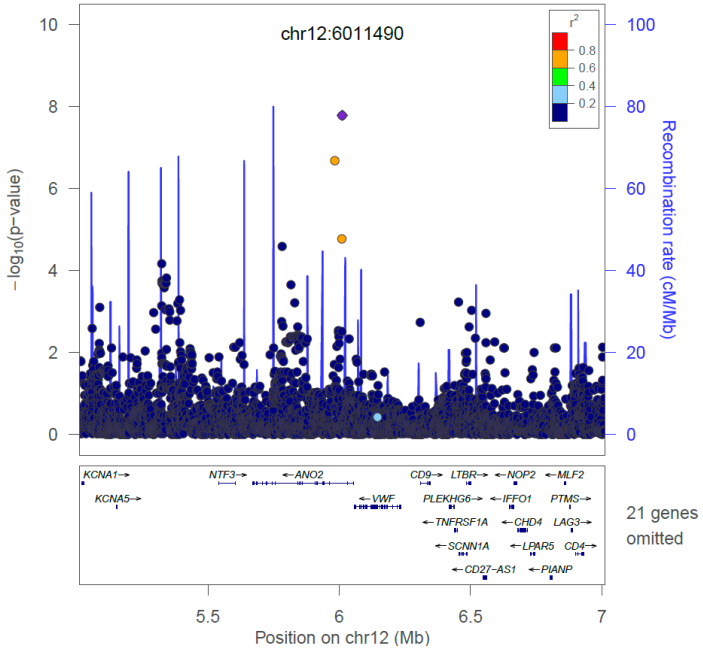
Supplementary Figures and Tables

Supplemental Figure 1. Results of hormone GWAS: LocusZoom plots for significant signals (not all genes shown).(Linkage disequilibrium is based on 1000 Genomes Nov 2010 EUR; chr-pos is GRCh37/hg19.)

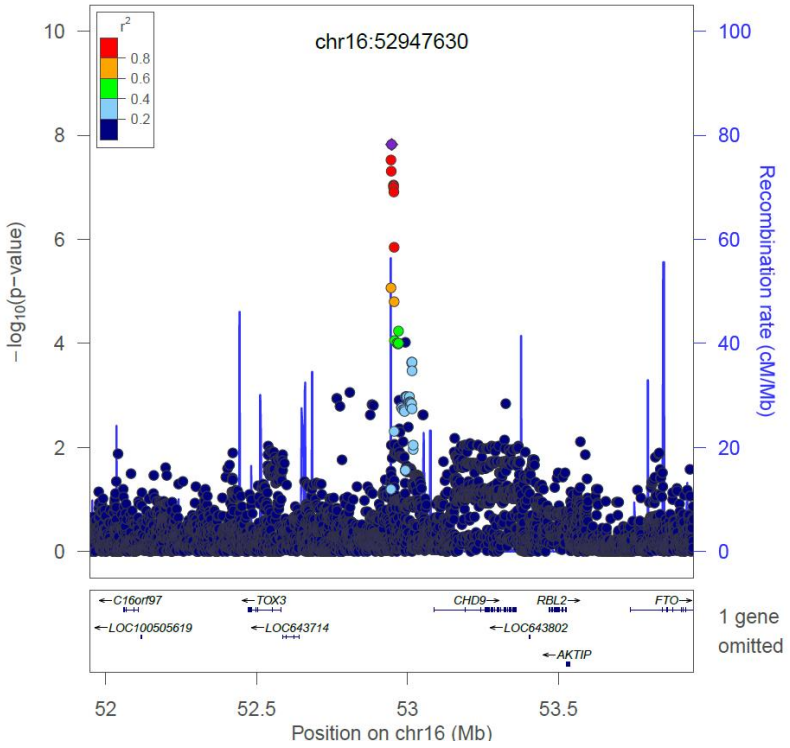
(a) DHEAS – rs148982377, chr7:99,075,038



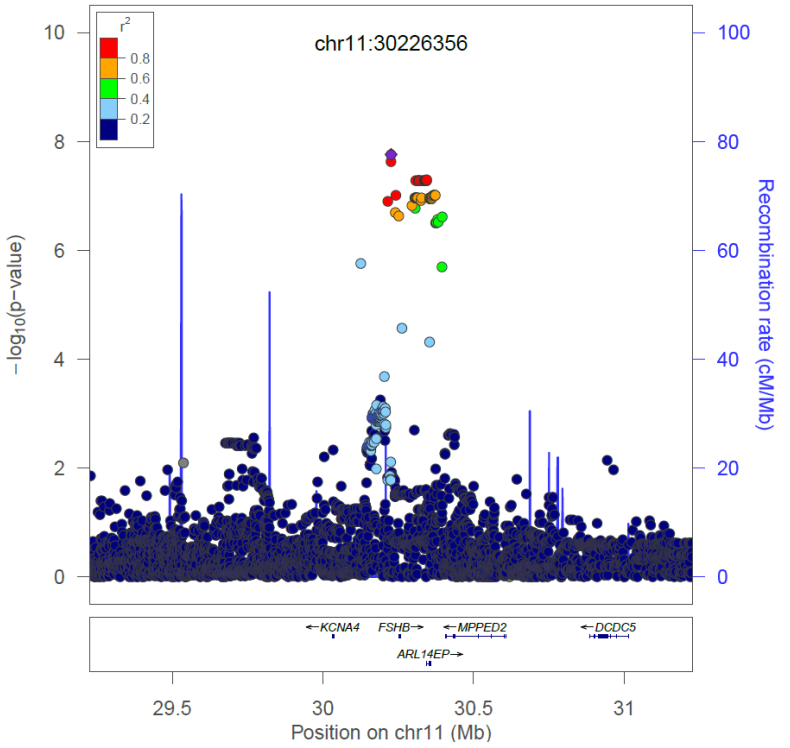
(b) Oestradiol – rs117585797, chr12:6,011,490



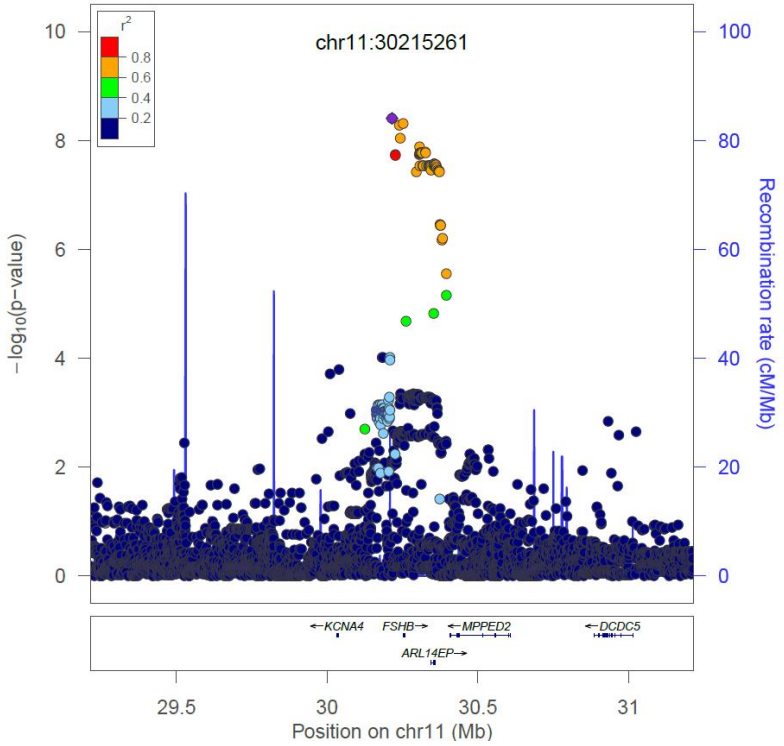
(c) FAI – rs117145500, chr16:52,947,630



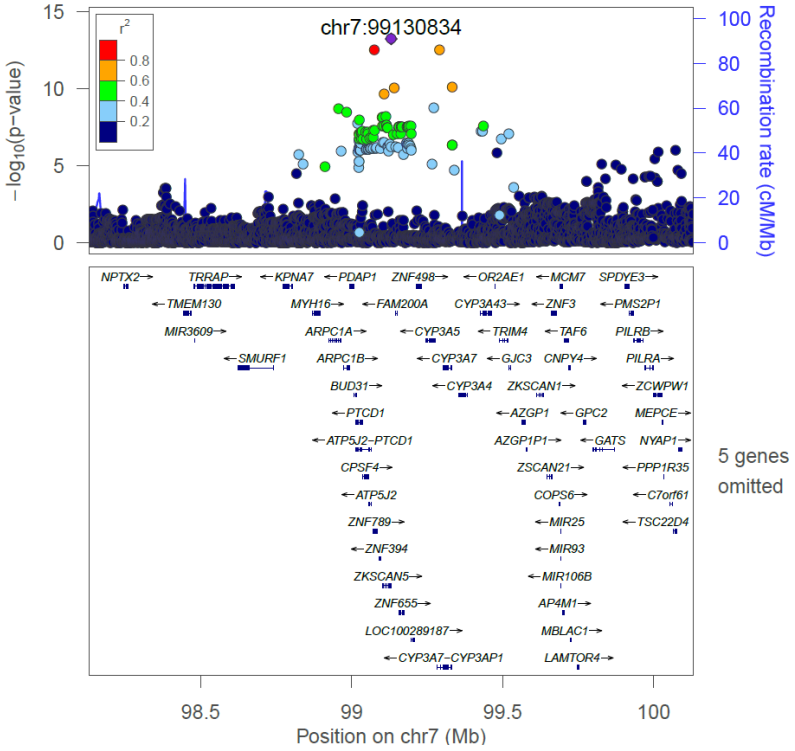
(d) FSH – rs11031005, chr11:30,226,356



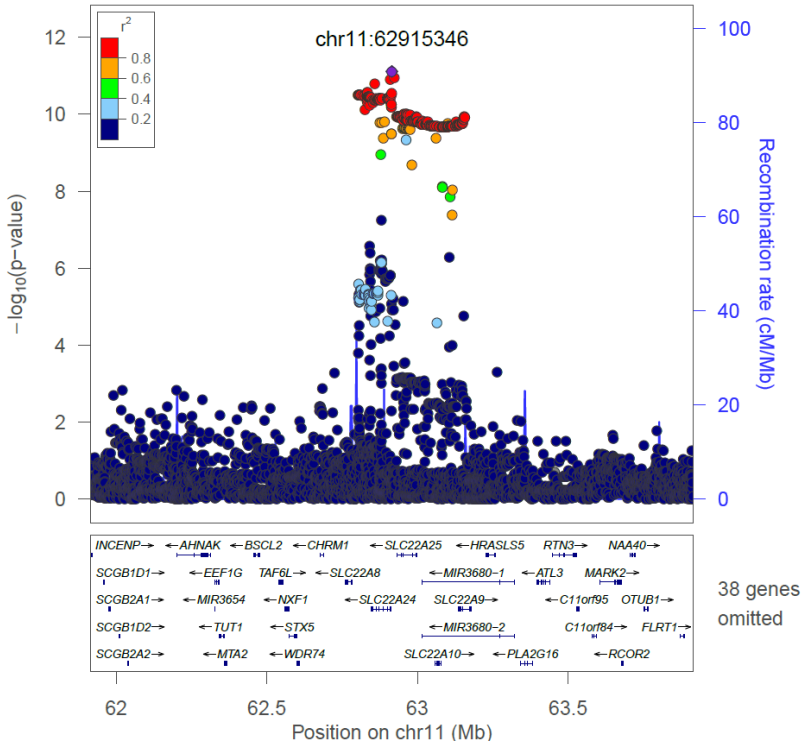
(e) LH – rs11031002, chr11:30,215,261



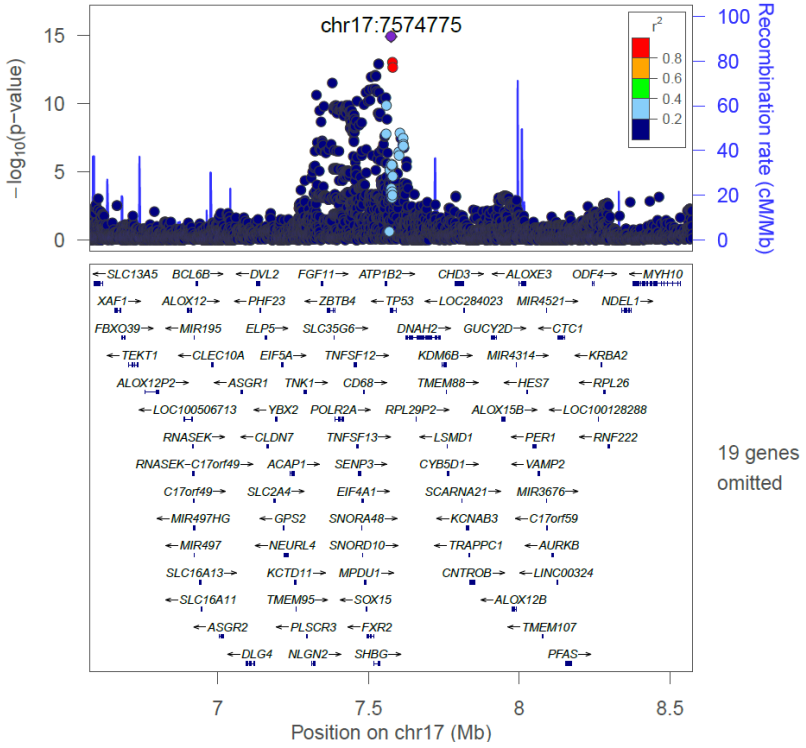
(f) Progesterone, chromosome 7 – rs34670419, chr7:99,130,834



(g) Progesterone, chromosome 11 – rs112295236, chr11:62,915,346

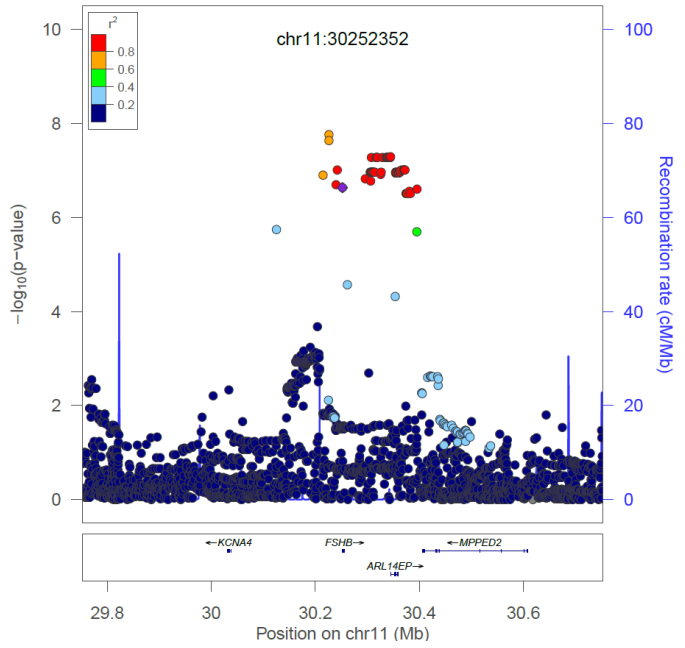


(h) SHBG – rs1641549, chr17:7,574,775

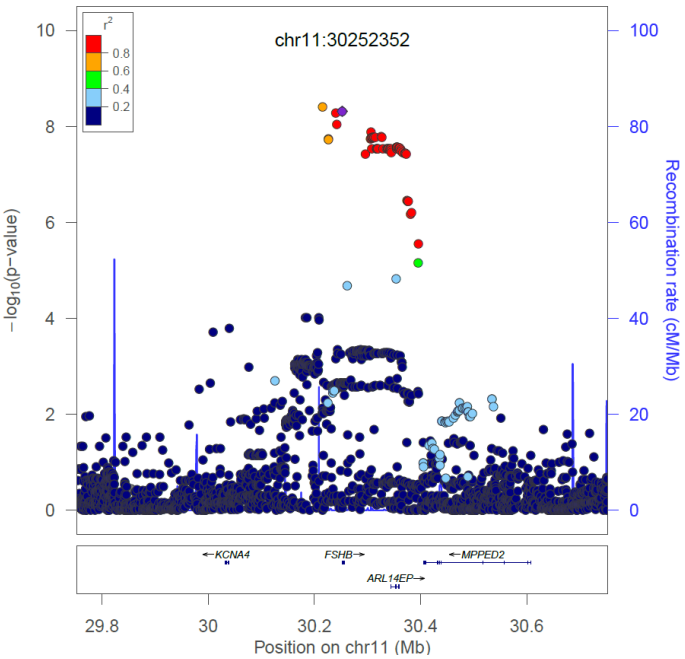


Supplemental Figure 2. LocusZoom plots for the Twins UK FSH and LH GWAS results showing linkage disequilibrium with the known FSHB promoter polymorphism (-211 G→T) rs10835638 (chr11.hg19:g. 30252352 G>T) (shown in purple). (Linkage disequilibrium is based on 1000 Genomes Nov 2010 EUR.)

(a) Twins UK FSH GWAS



(b) Twins UK LH GWAS



Supplementary Table 1. Descriptive statistics for cohort.

		% (N=2,913)
Sex	Male	10.17
	Female	89.83
Menstrual phase		
	Follicular	13.86
	Luteal	9.1
	Ovulatory	2.83
	Perimenopausal	11.14
	Postmenopausal	52.9
	N/a (male)	10.17

	Mean (standard deviation)	Range	Median (Lower quartile, upper quartile)
Age (years)	53.8 (12.5)	(16,82)	55 (46,62)
Height (m)	1.63 (0.07)	(1.41,2.05)	1.63 (1.58,1.67)
Weight (kg)	70.6 (14)	(37.9,146.5)	68 (60.5,78)
BMI (kg/m^2)	26.6 (5)	(15.8,53.8)	25.6 (23.2,29.1)

Supplemental Table 2: Summary of values in the Twins UK hormone analyses for published genetic variants associated with reproductive hormones (autosomal variants only)[2,4,7-10]. Green highlighting indicates effect in same direction for Twins UK study and published, orange highlighting indicates effect in opposite direction.

a) DHEAS, FAI, FSH, LH, oestradiol (continued on next page).

Published study					**DHEAS**		**FAI**		**FSH**		**LH**		**Oestradiol**	
Chr-position (GRCh37/hg19)	**Study**	**SNP id**	**Near gene**	**Dir. effect**	**Beta**	**P-value**	**Beta**	**P-value**	**Beta**	**P-value**	**Beta**	**P-value**	**Beta**	**P-value**
10-94485211	Zhai (DHEAS)	rs2497306	*HHEX*	+	3.37E-02	1.55E-02	3.47E-02	1.28E-01	2.59E-02	3.55E-01	1.57E-02	5.23E-01	-7.75E-03	6.99E-01
10-96751270	Zhai (DHEAS)	rs2185570	*CYP2C9*	+	2.63E-02	1.95E-01	2.08E-02	5.35E-01	-2.91E-02	4.76E-01	-5.81E-02	1.04E-01	6.01E-03	8.37E-01
15-40360741	Zhai (DHEAS)	rs7181230	*BMF*	-	-4.46E-02	1.86E-03	1.26E-03	9.57E-01	5.94E-03	8.39E-01	-3.75E-02	1.44E-01	-2.71E-02	1.95E-01
19-48401893	Zhai (DHEAS)	rs2637125	*SULT2A1*	+	8.89E-02	1.91E-06	8.59E-03	7.80E-01	-2.21E-02	5.63E-01	-6.45E-02	5.48E-02	-6.99E-02	1.02E-02
2-111949327	Zhai (DHEAS)	rs6738028	*BCL2L11*	-	-4.94E-02	8.10E-04	-2.05E-02	4.00E-01	2.68E-02	3.69E-01	3.24E-02	2.15E-01	-2.13E-02	3.16E-01
7-98957880	Zhai (DHEAS)	rs740160	*ARPC1A*	-	1.45E-02	6.59E-01	3.37E-02	5.40E-01	-1.82E-02	7.88E-01	4.20E-02	4.75E-01	1.06E-01	2.84E-02
7-99118801	Zhai (DHEAS)	rs11761528	*ZKSCAN5*	+	**1.42E-01**	**1.79E-09**	7.82E-02	4.78E-02	-2.05E-02	6.70E-01	1.07E-02	7.98E-01	8.35E-02	1.52E-02
7-99489571	Zhai (DHEAS)	rs17277546	*TRIM4; CYP3A43*	+	5.90E-02	2.86E-03	1.01E-03	9.76E-01	1.17E-02	7.75E-01	3.59E-02	3.14E-01	-6.71E-02	2.20E-02
15-51524292	Chen(FSH)	rs2414095	*CYP19A1*	+	4.19E-03	7.76E-01	1.94E-02	4.18E-01	-3.67E-03	9.01E-01	-1.63E-02	5.30E-01	-7.52E-02	3.96E-04
15-51524292	Chen (Oestradiol)	rs2414095	*CYP19A1*	-	4.19E-03	7.76E-01	1.94E-02	4.18E-01	-3.67E-03	9.01E-01	-1.63E-02	5.30E-01	-7.52E-02	3.96E-04
15-51617708	Chen (Oestradiol)	rs2445762	*CYP19A1*	+	4.97E-02	9.62E-04	9.21E-02	2.11E-04	-3.46E-02	2.56E-01	-3.57E-02	1.82E-01	3.71E-02	8.87E-02
17-7487108	Chen (SHBG)	rs2075230	*SHBG*	+	3.58E-02	7.16E-02	-4.01E-02	2.25E-01	-1.37E-02	7.36E-01	2.64E-02	4.59E-01	5.03E-02	8.32E-02
10-65138910	Coviello (SHBG)	rs7910927	*JMJD1C*	-	-1.95E-02	1.64E-01	5.09E-02	2.62E-02	-3.29E-02	2.43E-01	-1.56E-02	5.29E-01	1.98E-02	3.26E-01
1-107546375	Coviello (SHBG)	rs17496332	*PRMT6*	-	-7.18E-03	6.24E-01	5.48E-05	9.98E-01	1.26E-02	6.72E-01	-1.78E-02	4.95E-01	-1.30E-02	5.40E-01
12-21331549	Coviello (SHBG)	rs4149056	*SLCO1B1*	+	-5.87E-02	2.20E-03	-3.19E-02	3.09E-01	8.22E-02	3.49E-02	2.33E-02	4.95E-01	-1.98E-02	4.76E-01
15-96708291	Coviello (SHBG)	rs8023580	*NR2F2*	-	-2.11E-02	1.71E-01	9.66E-03	7.04E-01	-4.17E-02	1.90E-01	-3.43E-02	2.18E-01	-4.57E-02	4.35E-02
17-47445751	Coviello (SHBG)	rs2411984	*ZNF652*	-	4.28E-03	7.70E-01	5.47E-02	2.32E-02	2.21E-02	4.57E-01	3.12E-02	2.33E-01	5.72E-03	7.87E-01

a) DHEAS, FAI, FSH, LH, oestradiol (continued from previous page).

Published study					DHEAS		FAI		FSH		LH		Oestradiol	
Chr-position (GRCh37/hg19)	**Study**	**SNP id**	**Near gene**	**Dir. effect**	**Beta**	**P-value**	**Beta**	**P-value**	**Beta**	**P-value**	**Beta**	**P-value**	**Beta**	**P-value**
17-7521915	Coviello (SHBG)	rs12150660	*SHBG*	-	2.60E-03	8.69E-01	7.82E-02	2.55E-03	2.05E-02	5.21E-01	7.75E-03	7.82E-01	-3.66E-02	1.06E-01
2-27742603	Coviello (SHBG)	rs780093	*GCKR*	-	5.40E-03	7.05E-01	-8.72E-03	7.09E-01	2.08E-02	4.68E-01	2.14E-02	3.95E-01	-3.08E-02	1.32E-01
2-48646399	Coviello (SHBG)	rs10454142	*LHCGR*	+	-2.45E-03	8.76E-01	1.95E-02	4.46E-01	6.34E-02	4.48E-02	6.16E-02	2.63E-02	1.69E-02	4.53E-01
4-69591782	Coviello (SHBG)	rs293428	*UGT2B15*	-	9.03E-03	5.53E-01	3.46E-02	1.68E-01	3.49E-02	2.65E-01	3.65E-02	1.84E-01	-2.26E-02	3.11E-01
7-97993362	Coviello (SHBG)	rs3779195	*BAIAP2L1*	+	-2.49E-02	1.73E-01	-8.96E-02	2.82E-03	-1.30E-02	7.27E-01	-1.42E-02	6.64E-01	2.37E-02	3.72E-01
8-81461974	Coviello (SHBG)	rs440837	*ZBTB10*	-	-1.28E-02	4.32E-01	8.57E-03	7.49E-01	5.26E-02	1.10E-01	3.97E-02	1.69E-01	-6.48E-02	5.84E-03
17-7537792	Prescott (SHBG)	rs727428	*SHBG*	-	-4.69E-03	7.46E-01	6.43E-02	6.84E-03	-1.92E-02	5.13E-01	6.41E-03	8.04E-01	5.46E-04	9.79E-01
17-7487108	Chen (Testosterone)	rs2075230	*SHBG*	+	3.58E-02	7.16E-02	-4.01E-02	2.25E-01	-1.37E-02	7.36E-01	2.64E-02	4.59E-01	5.03E-02	8.32E-02
10-65337153	Jin (Testosterone)	rs10822184	*JMJD1C*	-	-2.17E-02	1.25E-01	5.16E-02	2.57E-02	-3.04E-02	2.87E-01	-2.19E-02	3.82E-01	7.17E-03	7.25E-01
17-7537792	Jin (Testosterone)	rs727428	*SHBG*	-	-4.69E-03	7.46E-01	6.43E-02	6.84E-03	-1.92E-02	5.13E-01	6.41E-03	8.04E-01	5.46E-04	9.79E-01
17-7521915	Ohlsson (Testosterone)	rs12150660	*SHBG*	-	2.60E-03	8.69E-01	7.82E-02	2.55E-03	2.05E-02	5.21E-01	7.75E-03	7.82E-01	-3.66E-02	1.06E-01
17-7534678	Ohlsson (Testosterone)	rs6258	*SHBG*	+	-2.39E-02	7.48E-01	-4.01E-01	1.34E-03	-4.92E-02	7.50E-01	-1.21E-02	9.29E-01	-1.16E-01	2.90E-01

b) Progesterone, prolactin, SHBG, testosterone.

Published study					Progesterone		Prolactin		SHBG		Testosterone	
Chr-position (GRCh37/hg19)	**Study**	**SNP id**	**Near gene**	**Dir. effect**	**Beta**	**P-value**	**Beta**	**P-value**	**Beta**	**P-value**	**Beta**	**P-value**
10-94485211	Zhai (DHEAS)	rs2497306	*HHEX*	+	5.09E-02	4.94E-03	-2.60E-03	8.22E-01	-1.28E-02	3.35E-01	2.37E-02	1.47E-01
10-96751270	Zhai (DHEAS)	rs2185570	*CYP2C9*	+	1.45E-03	9.56E-01	-2.68E-02	1.12E-01	-1.27E-02	5.11E-01	-8.98E-04	9.70E-01
15-40360741	Zhai (DHEAS)	rs7181230	*BMF*	-	7.74E-04	9.67E-01	1.36E-02	2.59E-01	-2.91E-02	3.47E-02	-1.81E-02	2.85E-01
19-48401893	Zhai (DHEAS)	rs2637125	*SULT2A1*	+	1.34E-02	5.87E-01	-6.52E-03	6.80E-01	3.10E-03	8.63E-01	1.44E-02	5.16E-01
2-111949327	Zhai (DHEAS)	rs6738028	*BCL2L11*	-	-5.84E-02	2.47E-03	-4.09E-03	7.41E-01	7.29E-05	9.96E-01	-1.73E-02	3.22E-01
7-98957880	Zhai (DHEAS)	rs740160	*ARPC1A*	-	1.18E-02	7.89E-01	-1.52E-02	5.84E-01	-3.57E-02	2.59E-01	4.32E-03	9.13E-01
7-99118801	Zhai (DHEAS)	rs11761528	*ZKSCAN5*	+	1.75E-01	**3.34E-08**	-1.02E-02	6.05E-01	1.59E-02	4.80E-01	1.05E-01	2.18E-04
7-99489571	Zhai (DHEAS)	rs17277546	*TRIM4;CYP3A43*	+	6.32E-02	1.72E-02	6.39E-03	7.03E-01	3.09E-02	1.08E-01	2.90E-02	2.32E-01
15-51524292	Chen(FSH)	rs2414095	*CYP19A1*	+	-1.02E-02	5.92E-01	-2.06E-02	9.44E-02	-1.49E-02	2.89E-01	-7.60E-03	6.59E-01
15-51524292	Chen (Oestradiol)	rs2414095	*CYP19A1*	-	-1.02E-02	5.92E-01	-2.06E-02	9.44E-02	-1.49E-02	2.89E-01	-7.60E-03	6.59E-01
15-51617708	Chen (Oestradiol)	rs2445762	*CYP19A1*	+	4.53E-02	2.14E-02	1.06E-02	4.00E-01	-1.67E-02	2.47E-01	7.16E-02	6.15E-05
17-7487108	Chen (SHBG)	rs2075230	*SHBG*	+	3.25E-03	9.01E-01	7.16E-03	6.70E-01	7.47E-02	9.48E-05	5.68E-02	1.67E-02
10-65138910	Coviello (SHBG)	rs7910927	*JMJD1C*	-	-1.56E-02	3.91E-01	1.85E-02	1.13E-01	-4.86E-02	2.93E-04	-1.35E-02	4.13E-01
1-107546375	Coviello (SHBG)	rs17496332	*PRMT6*	-	-2.03E-03	9.16E-01	-2.04E-03	8.68E-01	-3.59E-03	7.98E-01	-2.20E-03	8.99E-01
12-21331549	Coviello (SHBG)	rs4149056	*SLCO1B1*	+	-3.45E-02	1.68E-01	2.08E-03	8.97E-01	9.83E-03	5.91E-01	-2.80E-02	2.14E-01
15-96708291	Coviello (SHBG)	rs8023580	*NR2F2*	-	-3.90E-02	5.63E-02	-1.83E-02	1.60E-01	-4.16E-02	5.12E-03	-2.44E-02	1.82E-01
17-47445751	Coviello (SHBG)	rs2411984	*ZNF652*	-	-4.06E-03	8.33E-01	-5.62E-03	6.48E-01	-4.70E-02	7.97E-04	-2.22E-03	8.98E-01
17-7521915	Coviello (SHBG)	rs12150660	*SHBG*	-	-7.75E-03	7.07E-01	1.07E-02	4.16E-01	-1.08E-01	7.92E-13	-4.60E-02	1.33E-02
2-27742603	Coviello (SHBG)	rs780093	*GCKR*	-	2.22E-02	2.28E-01	-1.91E-02	1.07E-01	-3.94E-02	3.72E-03	-2.97E-02	7.60E-02
2-48646399	Coviello (SHBG)	rs10454142	*LHCGR*	+	-2.47E-02	2.28E-01	1.37E-02	2.95E-01	-3.69E-02	1.38E-02	-1.70E-02	3.56E-01
4-69591782	Coviello (SHBG)	rs293428	*UGT2B15*	-	-1.28E-02	5.25E-01	-1.13E-02	3.81E-01	-1.40E-02	3.39E-01	1.59E-02	3.79E-01
7-97993362	Coviello (SHBG)	rs3779195	*BAIAP2L1*	+	-1.14E-02	6.35E-01	-3.41E-02	2.65E-02	6.60E-02	1.64E-04	-1.96E-02	3.61E-01
8-81461974	Coviello (SHBG)	rs440837	*ZBTB10*	-	3.14E-03	8.82E-01	-8.55E-04	9.50E-01	-4.88E-02	1.70E-03	-4.08E-02	3.34E-02
17-7537792	Prescott (SHBG)	rs727428	*SHBG*	-	-7.50E-03	6.93E-01	1.27E-02	2.95E-01	-8.67E-02	4.76E-10	-2.99E-02	8.11E-02
17-7487108	Chen (Testosterone)	rs2075230	*SHBG*	+	3.25E-03	9.01E-01	7.16E-03	6.70E-01	7.47E-02	9.48E-05	5.68E-02	1.67E-02
10-65337153	Jin (Testosterone)	rs10822184	*JMJD1C*	-	-1.90E-02	3.01E-01	1.15E-02	3.29E-01	-4.58E-02	7.37E-04	-6.76E-03	6.84E-01
17-7537792	Jin (Testosterone)	rs727428	*SHBG*	-	-7.50E-03	6.93E-01	1.27E-02	2.95E-01	-8.67E-02	**4.76E-10**	-2.99E-02	8.11E-02
17-7521915	Ohlsson (Testosterone)	rs12150660	*SHBG*	-	-7.75E-03	7.07E-01	1.07E-02	4.16E-01	-1.08E-01	**7.92E-13**	-4.60E-02	1.33E-02
17-7534678	Ohlsson (Testosterone)	rs6258	*SHBG*	+	-3.16E-02	7.51E-01	-4.38E-02	4.92E-01	4.83E-01	**1.45E-11**	5.13E-02	5.72E-01

Note: There was evidence of consistency of effects for DHEAS (7/8 same direction, p=0.04), SHBG (12/13 same direction, p=0.002) and testosterone (5/5 same direction, p=0.03). No evidence of consistency for oestradiol (2/2 same direction, p=0.25) and FSH (0/1 same direction, p=0.5).

Supplemental Table 3. Effect sizes and p-values for the significant signals in the other hormones in the Twins UK hormone GWAS.

		Significant signals identified in Twins UK GWAS – hormone and chr-position							
Values of the significant signal in the other Twins UK hormone GWAS results		**DHEAS** chr7.hg19: g.99075038 T>C, rs148982377	**FAI** chr16.hg19: g.52947630 A>C, rs117145500	**FSH** chr11.hg19: g.30226356 T>C, rs11031005	**LH** chr11.hg19: g.30215261 T>A, rs11031002	**Oestradiol** chr12.hg19: g.6011490 C>A, rs11758579	**Progesterone** chr7.hg19: g.99130834 G>T, rs34670419	**Progesterone** chr11.hg19: g.62915346 C>G, rs112295236	**SHBG** chr17.hg19: g.7574775 C>T, rs1641549
DHEAS	Effect	-0.255	**-0.103**	0.027	0.023	0.127	**-0.259**	0.054	-0.025
	P-value	1.82E-14	**4.29E-04**	1.76E-01	2.76E-01	9.02E-02	**1.97E-14**	5.66E-02	1.30E-01
FAI	Effect	-0.093	-0.276	0.02	0.021	0.11	-0.101	0.082	0.064
	P-value	9.79E-02	1.50E-08	5.48E-01	5.51E-01	3.73E-01	7.61E-02	8.20E-02	1.89E-02
FSH	Effect	0.018	0.071	-0.232	**-0.226**	-0.017	0.038	0.053	0.052
	P-value	7.97E-01	2.36E-01	1.74E-08	**1.24E-07**	9.14E-01	5.83E-01	3.60E-01	1.22E-01
LH	Effect	-0.019	0.11	**0.203**	0.221	0.103	-0.015	-0.069	0.026
	P-value	7.56E-01	3.49E-02	**1.84E-08**	3.94E-09	4.52E-01	8.11E-01	1.75E-01	3.71E-01
Oestradiol	Effect	-0.071	-0.023	-0.048	-0.045	0.624	-0.084	-0.02	-0.065
	P-value	1.47E-01	5.81E-01	9.75E-02	1.40E-01	1.63E-08	8.99E-02	6.21E-01	6.63E-03
Prolactin	Effect	0.005	-0.034	-0.004	0.001	0.066	0.006	0.028	0.02
	P-value	8.49E-01	1.62E-01	8.06E-01	9.66E-01	3.03E-01	8.30E-01	2.42E-01	1.62E-01
Progesterone	Effect	**-0.331**	-0.093	0.012	0.008	0.129	-0.346	0.255	-0.033
	P-value	**2.99E-13**	1.67E-02	6.51E-01	7.71E-01	1.98E-01	6.09E-14	7.68E-12	1.27E-01
SHBG	Effect	-0.085	0.121	-0.018	-0.017	0.066	-0.079	-0.021	-0.127
	P-value	7.85E-03	**1.51E-05**	3.55E-01	4.04E-01	3.63E-01	1.52E-02	4.51E-01	**1.21E-15**
Testosterone	Effect	**-0.203**	-0.087	-0.002	-0.004	0.117	**-0.213**	0.039	**-0.071**
	P-value	**5.39E-07**	1.39E-02	9.22E-01	8.78E-01	1.85E-01	**2.33E-07**	2.44E-01	**2.73E-04**

Notes: Values in bold are significant at p<5.00×10-3 (p<0.05 adjusted for approximately 10 tests per significant signal).Effect sizes shown are for the minor allele.

Supplemental Table 4. Effect sizes and p-values in the progesterone GWAS for variants known to be associated with DHEAS from the meta-analysis of Zhai et al[4]. The effects from the Twins UK GWAS are for the effect alleles stated in Zhai et al[4].

DHEAS variants identified by Zhai et al[4]						**Values in Twins UK progesterone GWAS analysis**		
Chr.-position (GRCh37/hg19)	**SNP**	**Gene**	**Effect allele**	**Effect**	**P-value**	**Effect**	**Standard error**	**P-value**
7-99118801	rs11761528	*ZKSCAN5*	T	-0.16	3.15×10^{-36}	-0.175	0.031	**3.34×10^{-8}**
19-48401893	rs2637125	*SULT2A1*	A	-0.09	2.61×10^{-19}	-0.013	0.025	5.87×10^{-1}
15-40360741	rs7181230	*BMF*	G	0.05	5.44×10^{-11}	-0.001	0.019	9.67×10^{-1}
10-94485211	rs2497306	*HHEX*	C	-0.04	4.64×10^{-9}	-0.051	0.018	**4.94×10^{-3}**
10-96751270	rs2185570	*CYP2C9*	C	-0.06	2.29×10^{-8}	-0.001	0.026	9.56×10^{-1}

Chr.=chromosome; DHEAS=dihydroepiandrosterone sulphate; SHBG=sex-hormone binding globulin. Notes: The p-value for a binomial sign test of consistency of direction was p=0.19. Values in bold are significant at p<0.01 (p<0.05 adjusted for five tests).

Supplemental Table 5. Effect sizes and p-values for the significant progesterone variants identified by the Twins UK GWAS in the data from the DHEAS meta-analysis of Zhai et al[4] .

Progesterone signal in Twins UK GWAS	**SNP id**	**Minor allele effect (% of s.d.)**	**Proxy in Zhai et al[4]**	**r^2 (SNP and proxy)**	**Gene location**	**P-value in Zhai et al[4]**	**z-score meta-analysis Zhai et al[4]**
chr7.hg19: g.99130834G>T	rs34670419	-55.6	rs10278040	0.58	Near *CYP3A* genes	**2.34×10^{-34}**	-12.2
chr11.hg19: g.62915346C>G	rs112295236	41.0	rs1939768	1	Near *SLC22A9*	**1.53×10^{-4}**	3.8

Notes: Both progesterone signals (highlighted in bold) are significant at p<2.5×10^{-2} (p<0.05 adjusted for two tests).

Supplemental Table 6. Effect sizes and p-values for the significant variants identified by the Twins UK GWAS in the published GWAS of age at menopause[24].

Significant variant from Twins UK GWAS				**Value of proxy in age at menopause meta-analysis**					
Hormone GWAS	Significant variant	Effect minor allele	P-value	Proxy	Distance of proxy from variant (GRCh37/hg 19)	r^2 (proxy and variant)	Effect minor allele	P-value	Effect in same direction as Twins UK GWAS?
DHEAS	rs148982377	-0.25	1.82×10^{-14}	rs10278040	66,335	0.65	0.05	4.77×10^{-1}	n
FAI	rs117145500	-0.28	1.50×10^{-8}	rs9928588	2,464	0.54	0.11	5.05×10^{-2}	n
FSH	rs11031005	-0.23	1.74×10^{-8}	rs11031005	-	1.00	0.23	**8.30×10^{-8}**	n
LH	rs11031002	0.22	3.94×10^{-9}	rs11031002	-	1.00	0.25	**3.52×10^{-8}**	y
Oestradiol	rs117585797	0.62	1.63×10^{-8}	rs4764574	11,786	0.23	0.06	3.79×10^{-1}	y
Progesterone (chr 7)	rs34670419	-0.35	6.09×10^{-14}	rs10278040	10,539	0.56	0.05	4.77×10^{-1}	n
Progesterone (chr 11)	rs112295236	0.26	7.68×10^{-12}	rs1939768	637	1.00	0.03	6.88×10^{-1}	y
SHBG	rs1641549	-0.13	1.21×10^{-15}	rs1042522	4,697	0.88	0.06	9.17×10^{-2}	n

Note: Values in bold are significant at $p<6.25\times10^{-3}$ (p=0.05 adjusted for eight tests).

Supplemental Table 7. Effect sizes and p-values for the significant variants identified by the Twins UK GWAS in the published GWAS of age at menarche[26].

Significant variant from Twins UK GWAS				**Value of proxy in age at menarche meta-analysis**					
Hormone GWAS	Significant variant	Effect minor allele	P-value	Proxy	Distance of proxy from variant (GRCh37/hg 19)	r^2 (proxy and variant)	Effect minor allele	P-value	Effect in same direction as Twins UK GWAS?
DHEAS	rs148982377	-0.25	1.82×10^{-14}	rs10278040	66,335	0.65	0.01	5.02×10^{-1}	n
FAI	rs117145500	-0.28	1.50×10^{-8}	rs9928588	2,464	0.54	-0.01	4.12×10^{-1}	y
FSH	rs11031005	-0.23	1.74×10^{-8}	rs11031005	-	1.00	0.04	**2.74×10^{-5}**	n
LH	rs11031002	0.22	3.94×10^{-9}	rs11031002	-	1.00	0.04	**4.56×10^{-5}**	y
Oestradiol	rs117585797	0.62	1.63×10^{-8}	rs4764574	11,786	0.23	-0.03	8.78×10^{-2}	n
Progesterone (chr 7)	rs34670419	-0.35	6.09×10^{-14}	rs10278040	10,539	0.56	0.01	5.02×10^{-1}	n
Progesterone (chr 11)	rs112295236	0.26	7.68×10^{-12}	rs1939768	637	1.00	-0.01	4.01×10^{-1}	n
SHBG	rs1641549	-0.13	1.21×10^{-15}	rs1042522	4,697	0.88	-0.01	4.40×10^{-1}	y

Notes: Values in bold are significant at $p<6.25\times10^{-3}$ (p=0.05 adjusted for eight tests).

Supplemental Table 8. P-values of published menopause variants[24] in the Twins UK GWAS.

SNP ID	Chr-position (GRCh37/ hg19)	DHEAS	FAI	FSH	LH	Oestradiol	Progesterone	Prolactin	SHBG	Testosterone
rs4246511	1-39380385	8.22E-01	6.39E-01	1.10E-01	4.15E-01	4.21E-01	5.07E-01	7.76E-01	2.85E-01	6.85E-01
rs2303369	2-27715416	7.72E-01	5.59E-01	3.57E-01	2.29E-01	1.45E-01	4.35E-01	4.96E-01	3.81E-01	1.18E-01
rs10183486	2-171990971	7.91E-01	8.85E-01	7.47E-01	7.77E-01	7.32E-01	3.18E-01	7.63E-01	5.85E-01	4.17E-02
rs1635501	1-242040775	7.29E-01	3.17E-01	4.79E-01	5.77E-01	8.60E-01	1.11E-01	5.81E-01	8.15E-01	5.25E-01
rs4693089	4-84373622	2.31E-01	2.26E-01	2.86E-01	5.18E-02	5.48E-01	4.71E-01	2.85E-03	9.74E-01	7.91E-01
rs365132	5-176378574	3.20E-01	4.55E-01	9.96E-01	9.62E-01	9.74E-01	6.35E-01	9.88E-01	7.01E-01	5.09E-01
rs2153157	6-10897488	3.83E-01	8.58E-01	9.07E-01	5.63E-01	4.51E-01	4.77E-01	1.00E+00	8.98E-01	6.35E-01
rs1046089	6-31602967	9.27E-01	3.62E-01	8.86E-01	1.68E-01	4.75E-01	1.99E-01	7.79E-02	6.88E-01	4.01E-01
rs2517388	8-37977732	4.31E-01	1.08E-01	6.15E-01	4.77E-01	2.38E-01	8.21E-01	3.57E-01	3.36E-01	9.02E-02
rs12294104	11-30382899	6.14E-01	8.73E-01	**3.02E-07**	**6.25E-07**	1.53E-01	7.61E-01	6.44E-01	4.01E-01	8.88E-01
rs2277339	12-57146069	1.32E-01	2.39E-02	8.97E-01	9.59E-01	3.96E-01	6.15E-02	8.11E-01	1.60E-02	6.79E-01
rs4886238	13-61113739	2.43E-01	7.13E-01	3.87E-02	2.35E-01	3.98E-01	8.88E-02	5.21E-01	7.63E-02	6.42E-01
rs2307449	15-89863928	1.59E-01	9.56E-01	7.80E-01	2.82E-01	5.51E-01	3.02E-01	5.85E-01	5.12E-01	9.31E-01
rs10852344	16-12016919	4.53E-01	2.98E-01	7.91E-02	6.62E-02	4.14E-01	1.99E-01	2.54E-01	5.19E-01	1.22E-01
rs11668344	19-55833664	6.58E-01	5.96E-01	2.88E-01	6.97E-01	6.09E-01	2.30E-01	8.61E-02	9.81E-01	1.66E-01
rs12461110	19-56320663	6.41E-01	8.44E-01	2.63E-01	2.92E-01	4.60E-01	3.33E-01	1.69E-01	9.95E-01	8.23E-01
rs16991615	20-5948227	5.02E-01	2.82E-01	7.31E-01	5.26E-01	1.05E-01	2.55E-01	7.16E-01	7.28E-01	3.07E-02

Note: Values in bold are significant at $p<3.3\times10^{-4}$, calculated on the basis of nine tests at each of 17 published SNPs.

Supplemental Table 9. P-values of published menarche variants[26] in the Twins UK GWAS.

SNP ID	Chr-position (GRCh37/hg19)	DHEAS	FAI	FSH	LH	Oestradiol	Progesterone	Prolactin	SHBG	Testosterone
rs466639	1-165394882	7.89E-02	1.26E-01	1.03E-01	2.62E-01	7.12E-01	6.75E-01	9.27E-01	1.71E-01	6.20E-04
rs633715	1-177852580	1.05E-01	2.89E-01	9.31E-01	5.02E-01	8.33E-01	9.75E-02	9.85E-01	1.92E-01	2.55E-02
rs2947411	2-614168	9.88E-01	6.16E-01	5.64E-01	4.49E-01	7.03E-01	2.64E-01	4.96E-01	7.13E-01	4.44E-01
rs17268785	2-56592083	9.62E-01	6.05E-01	6.92E-01	7.77E-01	8.17E-01	1.98E-01	6.29E-01	7.17E-01	7.59E-01
rs17188434	2-157096776	9.45E-01	6.12E-01	2.94E-01	6.02E-01	4.46E-01	3.94E-01	6.45E-01	8.89E-01	4.08E-01
rs12617311	2-199632565	7.79E-01	8.80E-01	2.06E-01	4.13E-01	3.72E-01	3.62E-01	9.15E-01	3.04E-01	5.92E-01
rs7617480	3-49210732	8.63E-01	7.90E-01	8.54E-01	8.20E-01	2.40E-01	1.03E-01	7.20E-01	7.31E-01	5.30E-01
rs6762477	3-50093209	9.83E-01	7.05E-01	1.70E-01	1.05E-01	3.69E-01	9.32E-01	8.70E-01	1.10E-01	1.86E-01
rs7642134	3-86916882	1.10E-01	9.50E-01	2.34E-03	2.55E-01	8.07E-01	6.80E-02	1.06E-01	4.88E-01	7.19E-01
rs6438424	3-117574822	8.96E-01	1.30E-01	7.05E-02	2.07E-01	4.38E-01	9.49E-01	1.28E-01	5.17E-01	5.65E-01
rs6439371	3-132610752	1.81E-01	9.60E-02	3.93E-01	7.49E-01	7.41E-01	1.13E-01	6.17E-01	5.90E-01	8.20E-01
rs2002675	3-185629568	6.99E-02	8.17E-01	6.25E-01	3.59E-01	7.10E-01	6.08E-01	8.94E-01	6.98E-01	5.24E-01
rs13187289	5-133849177	6.49E-01	2.52E-01	9.41E-01	2.92E-01	9.92E-01	3.53E-01	2.16E-02	8.97E-01	7.02E-02
rs4840086	6-100208438	1.35E-01	3.62E-01	2.93E-01	2.41E-01	4.59E-01	7.57E-02	6.36E-01	7.70E-01	1.83E-01
rs7759938	6-105378954	6.83E-01	5.43E-02	9.97E-01	8.83E-01	4.89E-01	1.50E-01	7.82E-01	2.67E-02	9.63E-01
rs1361108	6-126767600	4.97E-01	4.44E-01	**1.50E-04**	4.80E-02	6.83E-01	2.86E-01	1.13E-02	5.24E-01	6.21E-01
rs1079866	7-41470093	3.88E-01	1.14E-01	4.70E-02	7.54E-02	6.40E-01	7.53E-01	1.40E-03	6.17E-01	5.76E-02
rs7821178	8-78093837	4.98E-01	8.63E-01	7.14E-01	5.39E-01	8.92E-01	6.43E-02	8.77E-01	2.90E-01	4.13E-01
rs2090409	9-108967088	9.40E-01	5.81E-01	3.13E-01	4.62E-01	5.32E-01	6.52E-01	8.12E-01	3.39E-01	8.03E-01
rs10980926	9-114293634	9.30E-01	1.09E-01	4.47E-01	5.03E-02	8.17E-01	3.83E-01	9.77E-02	3.87E-01	6.87E-01
rs4929923	11-8639200	8.20E-01	4.03E-01	1.66E-01	3.80E-02	6.04E-01	2.67E-01	2.73E-01	7.43E-02	7.98E-01
rs900145	11-13293905	1.96E-01	1.46E-01	7.90E-01	8.02E-01	8.03E-01	3.74E-01	1.12E-01	2.30E-01	4.71E-02
rs10899489	11-78095373	1.08E-01	8.43E-01	7.48E-01	7.77E-01	4.39E-01	5.12E-01	9.94E-01	6.40E-01	6.74E-01
rs6589964	11-122870683	6.40E-01	1.00E-01	6.77E-01	7.16E-01	1.05E-01	3.95E-01	6.61E-01	2.72E-01	3.34E-01
rs6575793	14-101032217	2.64E-01	9.76E-01	9.35E-01	2.08E-01	3.53E-01	6.00E-01	4.96E-01	9.15E-01	8.45E-01
rs1659127	16-14388305	7.49E-01	7.42E-01	5.67E-01	9.20E-02	7.46E-01	3.55E-01	2.06E-01	6.54E-01	9.15E-01
rs9939609	16-53820527	8.64E-01	2.31E-01	3.62E-01	9.54E-01	1.44E-01	8.31E-01	2.70E-01	4.69E-01	2.80E-01
rs1364063	16-69588572	2.90E-01	4.99E-01	5.11E-01	6.59E-01	8.67E-01	2.55E-01	2.08E-01	3.66E-01	9.25E-01
rs9635759	17-49613785	9.13E-01	8.58E-03	8.00E-01	2.97E-01	3.10E-01	5.14E-01	4.91E-01	2.10E-01	1.02E-01
rs1398217	18-44752238	6.89E-01	7.47E-01	1.12E-01	9.24E-01	5.92E-01	7.96E-01	1.14E-01	7.23E-01	4.53E-01
rs10423674	19-18817903	5.09E-01	6.90E-01	2.68E-01	1.52E-01	6.30E-01	1.97E-01	9.76E-01	1.27E-01	1.80E-01
rs852069	20-17122593	6.03E-01	8.10E-01	5.38E-01	4.30E-01	2.43E-01	9.58E-01	6.91E-01	6.71E-01	6.39E-01

Note: Values in bold are significant at p<1.74×10-4, calculated on the basis of nine tests at 32 published SNPs.

Supplemental Table 10. Candidate genes and expression qualitative trait loci (eQTL) associated with the significant signals.

Hormone	Chr-position	SNP id	P-value	Location of SNP	eQTL (Proxy for signal r^2>0.8 with associations)	Other genes within 300kb from start/end of gene
DHEAS	chr7.hg19:g.99075038T>C	rs148982377T>C	1.82×10^{-14}	*ZNF789*	N/a[1]	*ARPC1A*, *ARPC1B*, *ATP5J2*, *ATP5J2-PTCD1*, *BUD31*, *CPSF4*, *CYP3A4*, *CYP3A5*, *CYP3A7*, *CYP3A7-CYP3AP1*, *FAM200A*, *KPNA7*, *LOC100289187*, *MHY*[2], *PDAP1*, *PTCD1*, *ZKSCAN5*, *ZNF394*[2], *ZNF655*, *ZNF789*, *ZSCAN25*
FAI	chr16.hg19:g.52947630A>C	rs117145500A>C	1.50×10^{-8}	intergenic	N/a[1]	*CHD9*, *LOC643714*
FSH	chr11.hg19:g.30226356T>C	rs11031005T>C	1.74×10^{-8}	intergenic	rs11031005 – No eQTL associations	*ARL14EP*[2], *FSHB*, *KCNA4*, *MPPED2*
LH	chr11.hg19:g.30215261T>A	rs11031002T>A	3.94×10^{-9}	intergenic	rs11031002 – No eQTL associations	*ARL14EP*[2], *FSHB*, *KCNA4*, *MPPED2*
Oestradiol	chr12.hg19:g.6011490C>A	rs117585797C>A	1.63×10^{-8}	*ANO2*	N/a[1]	*CD9*, *VWF*
Progesterone	chr7.hg19:g.99130834G>T	rs34670419G>T	6.09×10^{-14}	*ZKSCAN5*	N/a[1]	*ARPC1A*, *ARPC1B*, *ATP5J2*, *ATP5J2-PTCD1*, *BUD31*, *CPSF4*, *CYP3A4*, *CYP3A43*, *CYP3A5*, *CYP3A7*, *CYP3A7-CYP3AP1*, *FAM200A*, *LOC100289187*, *MHY*[2], *PDAP1*, *PTCD1*, *ZKSCAN5*, *ZNF394*[2], *ZNF655*, *ZNF789*, *ZSCAN25*
Progesterone	chr11.hg19:g.62915346C>G	rs112295236C>G	7.68×10^{-12}	intergenic	rs1939768 – No eQTL associations	*CHRM1*, *HRASLS5*, *MIR3680-1*[2], *MIR3680-2*[2], *SLC22A10*, *SLC22A24*, *SLC22A25*, *SLC22A6*, *SLC22A8*, *SLC22A9*, *SLC3A2*
SHBG	chr17.hg19:g.7574775C>T	rs1641549C>T	1.21×10-15	*TP53*	rs1042522 – *EFNB3* in adipose (*p*=9.9E-12) and skin (*p*=2.72E-05)	*ATP1B2*, *C17orf61-PLSCR3*, *C17orf74*, *CD68*, *CHD3*, *CHRNB1*, *CYB5D1*, *DNAH2*, *EFNB3*, *EIF4A1*, *FGF11*, *FXR2*, *KDM6B2*, *LSMD12*, *MPDU1*, *NLGN2*, *PLSCR3*, *POLR2A*, *RPL29P22*, *SAT2*, *SENP3*, *SENP3-EIF4A1*, *SHBG*, *SLC35G6*, *SLC35G6*, *SNORA482*, *SNORA672*, *SNORD102*, *SOX15*, *SPEM12*, *TMEM1022*, *TMEM256*, *TMEM88*, *TNFSF12*, *TNFSF12-TNFSF13*, *TNFSF13*, *TNK1*, *TP53*, *WRAP53*, *ZBTB4*

Notes: [1]N/a = Best proxy r^2<0.8; [2] Provisional.

Supplemental Table 11. Values of the known FSHB promoter polymorphism (-211 G→T) rs10835638 (chr11.hg19:g. 30252352 G>T) in the Twins UK FSH and LH GWAS results.

Hormone	Imputation quality	Effect allele frequency	Effect	P-value
FSH	0.983	0.857	0.209	2.31×10^{-7}
LH	0.983	0.857	-0.207	4.84×10^{-9}

Chapter 4:

Genetic evidence that lower circulating FSH levels lengthen menstrual cycle, increase age at menopause, and impact female reproductive health

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Main text

Abstract

Study question: How does a genetic variant altering follicle stimulating hormone (FSH) levels impact female reproductive health?

Summary answer: The T allele of the *FSHB* promoter polymorphism (rs10835638; c.-211G>T) results in longer menstrual cycles and later menopause and, while having detrimental effects on fertility, is protective against endometriosis.

What is known already: The *FSHB* promoter polymorphism (rs10835638; c.-211G>T) affects levels of *FSHB* transcription and, as a result, levels of FSH. FSH is required for normal fertility and genetic variants at the *FSHB* locus are associated with age at menopause and polycystic ovary syndrome (PCOS).

Study design, size, duration: We used cross-sectional data from the UK Biobank to look at associations between the *FSHB* promoter polymorphism and reproductive traits, and performed a genome-wide association analysis for length of menstrual cycle.

Participants/materials, setting, methods: We included white British individuals aged 40–69 years in 2006–2010, included in the May 2015 release of genetic data from UK Biobank. We tested the FSH lowering T allele of the *FSHB* promoter polymorphism (rs10835638; c.-211G>T) for associations with 29 mainly female reproductive phenotypes in up to 63,350 individuals. We conducted a genome-wide association study (GWAS) in 9,534 individuals to identify genetic variants associated with length of menstrual cycle.

Main results and the role of chance: The FSH-lowering T allele of the *FSHB* promoter polymorphism (rs10835638; MAF 0.16) was associated with longer menstrual cycles (0.16 s.d. (approx. 1 day) per minor allele; 95% CI 0.12–0.20; $P=6\times10^{-16}$), later age at menopause (0.13 years per minor allele; 95% CI 0.04-0.22; $P=5.7\times10^{-3}$), greater female nulliparity (OR=1.06; 95% CI 1.02-1.11; $P=4.8\times10^{-3}$) and lower risk of endometriosis (OR=0.79; 95% CI 0.69–0.90; $P=4.1\times10^{-4}$). The FSH-lowering T allele was not associated more generally with other female reproductive illnesses or conditions and we did not replicate

associations with male infertility or PCOS. In the GWAS for menstrual cycle length, only variants near the *FSHB* gene reached genome-wide significance ($P<5\times10^{-8}$).

Limitations, reasons for caution: The data included might be affected by recall bias. Women with a cycle length recorded were aged over 40 and were approaching menopause, however we did not find evidence that this affected the results. Many of the illnesses had relatively small sample sizes and so we may have been under-powered to detect an effect.

Wider implications of the findings: We found a strong novel association between a genetic variant that lowers FSH levels and longer menstrual cycles, at a locus previously robustly associated with age at menopause. The variant was also associated with nulliparity and endometriosis risk. We conclude that lifetime differences in circulating levels of FSH between individuals can influence menstrual cycle length and a range of reproductive outcomes, including menopause timing, infertility, endometriosis and PCOS.

Introduction

Follicle stimulating hormone (FSH) is a key pituitary expressed hormone, which stimulates maturation of oocytes and is a biomarker of ovarian reserve. FSH is a heterodimer comprised of a hormone specific β-chain (FSH-β) associated with an α-chain shared by other members of the glycoprotein hormone family [1]. The anterior pituitary produces FSH with transcription of *FSHB* rate-limiting for FSH production. FSH stimulates target cells by binding to the FSH receptor (FSHR), a G-protein coupled receptor [2], promoting follicle maturation and oestrogen production in women, and Sertoli cell proliferation and spermatogenesis in males [1].

Rare mutations in the *FSHB* gene cause truncation of the FSH-β protein and result in hypogonadism and primary amenorrhea in females [3-5] and, in a male, delayed puberty with azoospermia [6]. Mouse models suggest that FSH is required for normal fertility. Female *Fshb* knockout mice are infertile and fail to complete normal folliculogenesis while male knockouts remain fertile but have reduced sperm counts, and infertility is observed in both male and female transgenic mice overexpressing human FSH [7,8].

A polymorphism in the promoter of *FSHB* (rs10835638; c.-211G>T) -211 bp upstream of the transcription start site is associated with reduced FSH-β *in vitro* and in human genetic studies. *In vitro*, the T allele of the promoter polymorphism reduces expression of a luciferase reporter gene [9] and decreases *FSHB* transcription in gonadotrope cells as a result of reduced LHX3 homeodomain transcription factor binding [10]. The T allele of rs10835638 (c.-211G>T) is associated with lower FSH levels in males and females, and with higher LH and lower testicular volume, sperm count, FSH/LH ratio, inhibin B and testosterone in males, and has been found at a higher prevalence in infertile males [11-18]. Genetic association studies have identified signals at the *FSHB* locus associated with age at menopause [19,20], polycystic ovary syndrome (PCOS) [21] and levels of luteinising hormone (LH) [17,21].

Using the unique resource of the UK Biobank [22], we show that a common genetic variant known to alter FSH levels impacts a wide range of traits important to female reproductive health, including fertility, endometriosis and menstrual cycle length. In the first genome-wide association study for menstrual cycle length, we identified the *FSHB* locus as the only signal associated with this trait.

Methods

Source of data

The UK Biobank includes 503,325 people aged 40–69 years recruited in 2006–2010 from across the UK [22]. We analysed data from the May 2015 interim release of imputed genetic data from UK Biobank which contains 73,355,667 SNPs, short indels and large structural variants in 152,249 individuals (http://www.ukbiobank.ac.uk/wp-content/uploads/2014/04/imputation_documentation_May2015.pdf). UK Biobank invited 9.2 million people to participate, giving a response rate of 5.47% [23]. Participants were registered with the UK National Health Service and lived within 25 miles of one of the 22 assessment centres. Participants answered detailed questions about themselves, had measurements taken and provided samples. Two arrays with over 95% common marker content were used to genotype the individuals, with approximately 50,000 people genotyped

on the UK BiLEVE array, and the remainder genotyped on the UK Biobank Axiom array .

Phenotypes

We derived reproductive phenotypes from the UK Biobank data (Supplementary methods). Continuous phenotypes were age at birth of first and last child (females only), age at menarche, age at natural menopause, length of menstrual cycle, number of live births and number of children fathered (included to test the association with male fertility). Menstrual cycle length was only recorded in women still cycling and they were asked “How many days is your usual menstrual cycle? (The number of days between each menstrual period.)” (excluding those answering <7 or >365; and if the answer <12 or >60, then the participant was asked to confirm).

To test assumptions of linearity, we analysed the binary outcomes early menarche (lower 5% tail), early menopause (20–44 years), long menstrual cycle (>31 days), short menstrual cycle (<20 days), and multiple pregnancy loss (>1 case).

We defined two infertility-related binary phenotypes; never pregnant (females) and never fathered a child (males). We analysed female medical conditions as binary outcomes comparing people reporting a condition (case) with those who did not (control). Medical conditions included dysmenorrhea, endometriosis, fibroids, irregular menstrual cycles, menopausal symptoms, menorrhagia, ovarian cysts, polycystic ovary syndrome (PCOS), uterine polyps, vaginal/uterine prolapse and breast, endometrial and ovarian cancer. As more general indicators of gynaecological health, we included the medical interventions bilateral oophorectomy or hysterectomy in our analysis. Summary statistics for the reproductive traits are presented in Tables 1 and 2.

Table 1. Description of cohort of unrelated individuals for continuous outcome measures.

Phenotype	N	Min	Max	Mean	S.D.	Lower quartile	Median	Upper quartile
Age at first birth (years)[1]	43,066	10	50	25.1	4.6	22	25	28
Age at last birth (years) [1]	43,008	15	50	30.0	4.8	27	30	33
Age at menarche (years) [1]	61,306	9	17	12.9	1.6	12	13	14
Age at natural menopause (years) [1]	27,996	18	65	49.9	4.5	48	50	53
Length of menstrual cycle (days) [1]	8,870	7	300	26.8	6.2	25	28	28
Number of children fathered[2]	56,508	0	28	1.8	1.2	1	2	2
Number of live births[1]	63,306	0	22	1.8	1.2	1	2	2

[1]Females only; [2]Males only.

Table 2. Number of people included in binary outcome measures.

Phenotype	Description	Cases	Controls	N
Bilateral oophorectomy[1]	Yes vs no	5,118	57,177	62,295
Dysmenorrhea[1]	Yes vs none recorded	78	63,272	63,350
Breast cancer[1]	Breast cancer recorded on cancer registry vs none recorded	2,810	60,540	63,350
Early menarche[1]	Youngest 5% age at menarche vs oldest 5%	3,050	3,050	6,100
Early menopause[1]	Natural menopause at 20-45 years vs 50-60 years	3,058	17,805	20,863
Endometrial cancer[1]	Endometrial cancer recorded on cancer registry vs none recorded	342	63,008	63,350
Endometriosis[1]	Yes vs none recorded	993	62,357	63,350
Fibroids[1]	Yes vs none recorded	1,819	61,531	63,350
Hysterectomy[1]	Yes vs no	4,753	50,932	55,685
Irregular menstrual cycles[1]	Irregular menstrual cycles vs regular cycle	2,490	10,316	12,806
Long menstrual cycle (vs average)[1]	Menstrual cycle >31 days vs 28 days	237	3,889	4,126
Menopausal symptoms[1]	Yes vs none recorded	126	63,224	63,350
Menorrhagia[1]	Yes vs none recorded	348	63,002	63,350
Multiple pregnancy loss[1]	More than one pregnancy loss vs none	4,047	33,191	37,238
Never fathered child[2]	Never fathered a child vs one or more children fathered	11,729	44,779	56,508
Never pregnant[1]	Never pregnant vs one or more pregnancies	9,247	52,966	62,213
Ovarian cancer[1]	Ovarian cancer recorded on cancer registry vs none recorded	247	63,103	63,350
Ovarian cysts[1]	Yes vs none recorded	1,015	62,335	63,350
Polycystic ovary syndrome[1]	Yes vs none recorded	153	63,197	63,350
Short menstrual cycle (vs average)[1]	Menstrual cycle <20 days vs 28 days	288	3,889	4,177
Uterine polyps[1]	Yes vs none recorded	359	62,991	63,350
Vaginal/uterine prolapse[1]	Yes vs none recorded	653	62,697	63,350

[1]Females only; [2]Males only.

Participants

In our analysis, we included individuals who both self-identified as white British and were confirmed as ancestrally Caucasian by UK Biobank from genetic information (n=128,266). We calculated principal components (PCs) for inclusion as covariates in our analyses using FlashPCA [24]. PCs were calculated in 120,286 unrelated participants (as identified by UK Biobank) based on 95,535 independent, directly genotyped SNPs (pairwise r^2 <0.1). These SNPs had MAF≥2.5% and missing-ness<1.5% across all participants in the May 2015 interim release of genetic data, and had HWE $P>1\times10^{-6}$ within the white British participants.

Testing for associations of the FSHB promoter polymorphism with reproductive phenotypes

We tested the FSH lowering T allele of the FSHB promoter polymorphism (rs10835638; c.-211G>T) for associations with reproductive phenotypes (up to 63,350 individuals). SNP rs10835638 was well-imputed in the data (imputation quality 0.995; HWE P=0.16; missing rate=0.3%). All analyses were carried out in males or females as appropriate (based on self-defined sex) using Stata (v13).

For continuous phenotypes, we transformed the phenotype by adjusting for recruitment centre, age at recruitment and first five PCs prior to inverse-normalisation. We performed linear regression of imputed minor-allele dosages at SNP rs10835638 on transformed phenotype with genotyping chip as a covariate. We carried out sensitivity analysis of the effect of different transformations, e.g. inverse normalising the trait prior to calculating the residuals, however this did not materially affect our results. Since the data on length of menstrual cycle included a wide range of values (Supplementary Figures 1 and 2), we carried out analyses on cycles from 21–35 days and in women aged <45 and ≥45 years at recruitment. We validated our results for length of menstrual cycle by carrying out analyses in two randomly chosen, equally-sized groups. For age at menopause and age at menarche, we also ran analysis using the phenotype definition from ReproGen Consortium (www.reprogen.org) GWAS (untransformed age at menopause between 40 and

60 years not adjusted for age, untransformed age at menarche) to allow comparisons with published data [19,20,25].

For binary outcomes, we performed logistic regression of the phenotype on minor-allele dosages at SNP rs10835638 including the first five PCs, recruitment centre, age at recruitment and genotyping chip as covariates.

GWAS of length of menstrual cycle

We conducted a genome-wide association study (GWAS) to identify genetic variants associated with length of menstrual cycle (n=9,534) using BOLT-LMM to account for relatedness and population structure [26]. This allowed us to include related individuals who were excluded from the association analysis of the FSHB promoter polymorphism (Supplementary Table 1). We transformed length of menstrual cycle by adjusting for recruitment centre and age at recruitment prior to inverse-normalisation, and performed association testing while adjusting for genotype chip. We filtered results on imputation quality>0.4, Hardy-Weinberg equilibrium $P>1\times10^{-5}$, and minor allele frequency >0.1%, resulting in approximately 14 million variants that were tested.

Results

A common allele in the FSHB gene, known to lower FSH levels, is associated with longer length of menstrual cycle

The FSH lowering T allele of the FSHB promoter polymorphism (rs10835638; MAF 0.16) was associated with longer menstrual cycles (0.16 s.d. (approx. 1 day) per minor allele; 95% CI 0.12–0.20; $P=6\times10^{-16}$). Of the reproductive traits tested, length of menstrual cycle was the most strongly associated with rs10835638 (Figure 1). The SNP was also associated with cycle length when we dichotomised into women reporting a cycle length of less than 28 days compared to those reporting the average length of 28 days (OR=0.70; 95% CI 0.54–0.90; $P=5.1\times10^{-3}$) (Figure 1). There was no evidence for an association with longer than 28 days compared to the average (OR=1.16; 95% CI 0.92–1.47; P=0.21). Results remained consistent when we analysed cycle lengths of 21–35 days and when we split our analysis into women aged <45 or ≥45 years

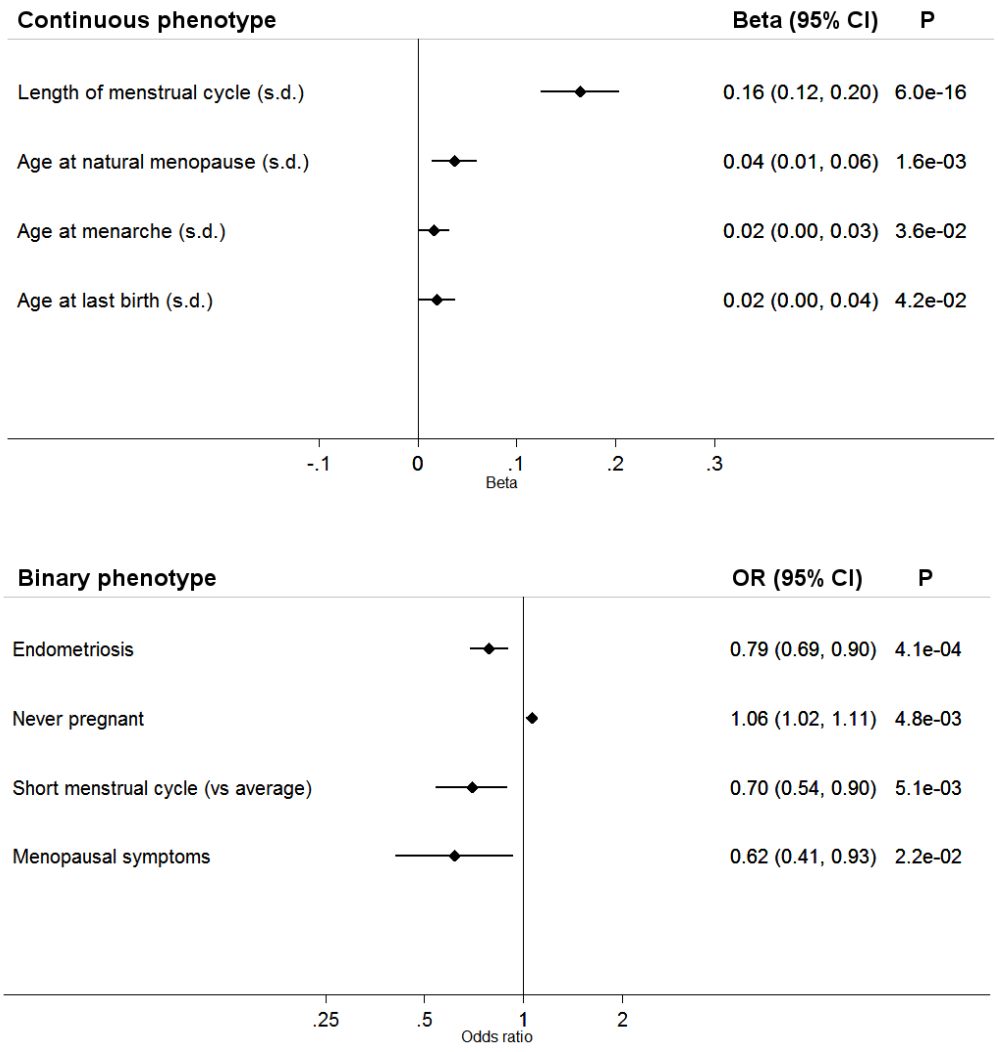
(Supplementary Figure 1). Analysis after randomly dividing the sample into two equal parts supported these results (Supplementary Figure 1).

Variants in or near the FSHB gene were the only ones that reached genome-wide significance in the GWAS for menstrual cycle length (Figure 2). The strongest association was for rs564036233G>GA, a 1 b.p. insertion which was associated with longer cycles by 1 day (0.16 s.d.) per minor allele (95% CI 0.12–0.20; $P=1.30\times10^{-16}$). The rs564036233 variant is in strong linkage disequilibrium (LD) with the promoter polymorphism rs10835638 ($r^2=0.82$) and conditional analysis indicated that rs564036233 and rs10835638 represent the same signal.

The FSHB allele associated with longer cycle length is associated with later menopause

The FSH lowering T allele of rs10835638 was associated with later age at menopause in UK Biobank (0.13 years per minor allele (ReproGen definition); 95% CI 0.04-0.22; $P=5.7\times10^{-3}$). There was no association between rs10835638 and menopause age when we dichotomised the phenotype into early menopause compared to later menopause (Table 3). The FSHB locus is known to be associated with timing of menopause: in GWAS conducted by the ReproGen consortium, the signal at this locus (rs12294104) increases age at menopause by 0.23 years (95% CI 0.16-0.29; $P=1.5\times10^{-11}$) [20]. Later menopause is associated with later age at last birth and rs10835638 was also associated with later age at last birth (0.02 s.d. (approx. 0.1 years) per T allele; 95% CI 0.00-0.04; $P=4.2\times10^{-2}$).

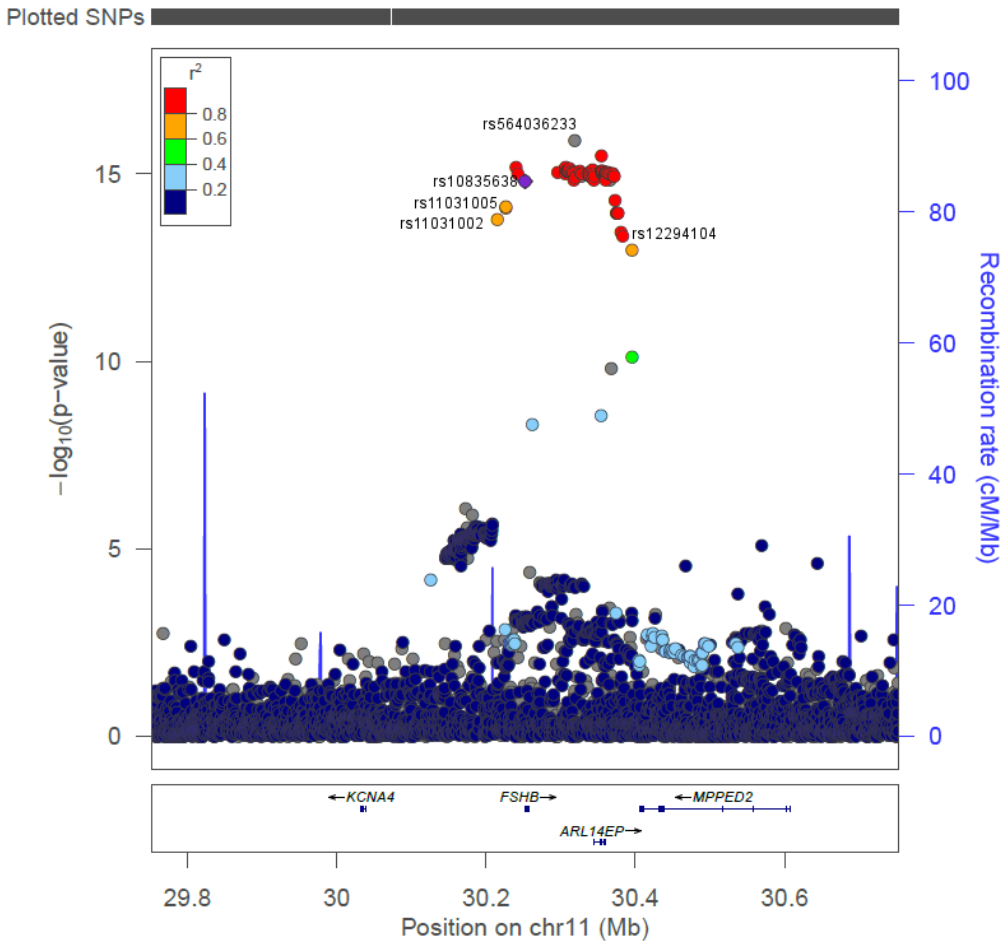
Figure 1. Phenotypes associated (p<0.05) with the FSH lowering allele of rs10835638 (c.-211G>T).



Notes: For continuous variables, effects (beta) are in standard deviations of the inverse-normally transformed variable to enable effect size comparisons.

CI=confidence interval; OR=odds ratio.

Figure 2. LocusZoom plot showing variants associated with length of menstrual cycle.



Notes:

The most strongly associated variant for cycle length is rs564036233. Linkage disequilibrium (1000 Genomes Nov 2014 EUR) shown is with rs10835638, the FSHB promoter polymorphism. Other SNPs indicated were the variants most significantly associated with FSHB (rs11031005) and LH (rs11031002) in a GWAS of hormone levels [17], and with age at natural menopause (rs12294104) in a meta-analysis [20].

Linkage disequilibrium values are not available for all SNPs since they are not included in 1000 Genomes Nov 2014 EUR.

Table 3. Associations with the FSH lowering T allele of rs10835638 (c.-211G>T).

Phenotype	Statistic	Effect(95% CI)	SE	P
Length of menstrual cycle (s.d.)	Beta	0.16(0.12,0.20)	0.02	**6.0E-16**
Endometriosis	OR	0.79(0.69,0.90)	0.05	4.1E-04
Age at natural menopause (s.d.)	Beta	0.04(0.01,0.06)	0.01	1.6E-03
Never pregnant	OR	1.06(1.02,1.11)	0.02	4.8E-03
Short menstrual cycle (vs average)	OR	0.70(0.54,0.90)	0.09	5.1E-03
Menopausal symptoms	OR	0.62(0.41,0.93)	0.13	2.2E-02
Age at menarche (s.d.)	Beta	0.02(0.00,0.03)	0.01	3.6E-02
Age at last birth (s.d.)	Beta	0.02(0.00,0.04)	0.01	4.2E-02
Age at first birth (s.d.)	Beta	0.02(0.00,0.03)	0.01	7.9E-02
Number of live births (s.d.)	Beta	-0.01(-0.03,0.00)	0.01	8.1E-02
Never fathered a child	OR	1.03(0.99,1.08)	0.02	1.2E-01
Early menopause	OR	0.95(0.88,1.02)	0.04	1.6E-01
Early menarche	OR	0.94(0.85,1.04)	0.05	2.1E-01
Fibroids	OR	0.94(0.86,1.03)	0.04	2.1E-01
Long menstrual cycle (vs average)	OR	1.16(0.92,1.47)	0.14	2.1E-01
Polycystic ovary syndrome	OR	1.18(0.88,1.59)	0.18	2.7E-01
Ovarian cysts	OR	0.94(0.83,1.07)	0.06	3.6E-01
Number of children fathered (s.d.)	Beta	0.01(-0.01,0.02)	0.01	4.1E-01
Menorrhagia	OR	0.92(0.74,1.13)	0.10	4.2E-01
Irregular menstrual cycles	OR	0.97(0.89,1.06)	0.04	4.6E-01
Multiple pregnancy loss	OR	0.98(0.91,1.04)	0.03	4.6E-01
Dysmenorrhea	OR	0.87(0.56,1.38)	0.20	5.6E-01
Breast cancer	OR	1.02(0.95,1.10)	0.04	6.4E-01
Ovarian cancer	OR	0.94(0.74,1.21)	0.12	6.4E-01
Vaginal/uterine prolapse	OR	0.97(0.83,1.13)	0.08	6.7E-01
Uterine polyps	OR	0.98(0.80,1.20)	0.10	8.6E-01
Endometrial cancer	OR	1.00(0.81,1.23)	0.11	9.7E-01

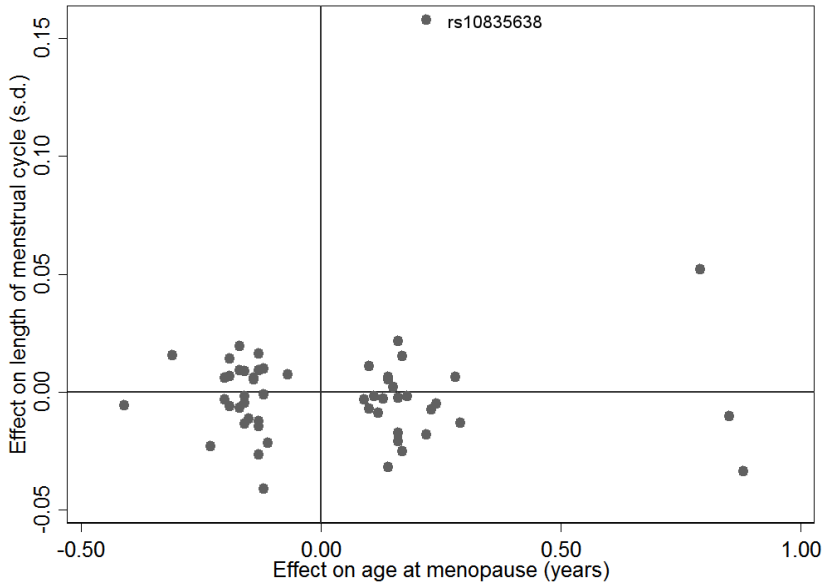
CI=confidence interval; OR=odds ratio; s.d.=standard deviations.

Note: For continuous variables, effects (beta) are in standard deviations of the inverse-normally transformed variable to enable effect size comparisons. Results significant at *P*<5E-08 are in bold; Results significant at *P*<5E-02 are underlined.

Longer cycle length is not a general feature of alleles associated with later age at menopause

We next tested the role of all other genetic variants associated with age at menopause. Only one of the other 55 published age at menopause signals was nominally associated with cycle length (P>0.05): rs10734411 was associated at p=0.005 [19,20,25]. For the 56 published menopause SNPs there was no correlation between the published effect estimates for age at menopause and the effect estimates from the GWAS for menstrual cycle length (R=0.064, p=0.63) (Figure 3). The FSHB SNP was an outlier in this plot, but removing it did not substantially affect the correlation (R=-0.027; P=0.84).

Figure 3. Comparison of the published effect size of the 56 known age at menopause variants [20,25] and their effect size in the GWAS for menstrual cycle length.



Notes: There was no significant correlation between the effects on age at menopause and cycle length (R= 0.064, p=0.63). The *FSHB* promoter polymorphism (rs10835638) is indicated.

The FSHB allele associated with lower FSH is also associated with an indicator of female infertility

The FSH lowering T allele of the FSHB promoter polymorphism (rs10835638) was associated with female nulliparity, i.e. greater odds of never being pregnant (OR=1.06; CI 1.02-1.11; P=4.8×10^{-3}) (Figure 1). The FSH lowering allele was not associated with other possible indicators of female infertility (later age at first birth and fewer live births) or male infertility (number of children fathered) (p>0.05) (Table 3).

The FSHB allele associated with higher FSH is also associated with higher odds of endometriosis and surgical intervention

The more common G allele was associated with increased odds of endometriosis (OR=1.27; CI 1.11-1.45; *P*=4.1×10^{-4}) (Figure 1). Of the seven published GWAS variants associated with endometriosis risk [27], the variant on chromosome 12 was nominally associated with cycle length, with the allele associated with an increased risk of endometriosis also associated with shorter cycles (p=0.02).

The G allele of rs10835638 was also associated with increased odds of having the medical interventions bilateral oophorectomy (OR=1.12; 95% CI 1.06-1.19; *P*=1.4×10^{-4}) and hysterectomy (OR=1.13; 95% CI 1.06-1.20; *P*=1.0×10^{-4}), which are used as treatments for a range of gynaecological conditions including endometriosis.

The common FSHB variant, associated with FSH levels, is not associated with reproductive traits more generally

There was no consistent evidence that the *FSHB* variant was associated with age at menarche. There was a 0.03 year increase in age at menarche (*ReproGen* definition) per T allele of rs10835638 (95% CI 0.01-0.05; *P*=1.4×10^{-2}) and the binary phenotype of early menarche was associated at p>0.05 (Table 3). None of 122 published GWAS signals for menarche [28] were associated with length of menstrual cycle at p<0.008.

The *FSHB* promoter polymorphism (rs10835638) was not associated with other reproductive illnesses or conditions at *P*<0.05 (Table 3), except for menopausal

symptoms (OR=0.62; 95% CI 0.41-0.93; p=0.02) (Figure 1). No associations were found with dysmenorrhea, fibroids, irregular menstrual cycles, menorrhagia, multiple pregnancy loss, ovarian cysts, PCOS, uterine polyps or vaginal/uterine prolapse, or with female breast, ovarian or endometrial cancer.

Discussion

In the first GWAS of menstrual cycle length we found a strong association between an FSH lowering, likely functional, variant in the *FSHB* promoter and longer cycles [9-14,16,17]. This locus has been previously robustly associated with age at menopause in the *ReproGen* consortium GWAS of menopause timing [19,20] and the allele associated with longer cycle length is associated with later age at menopause. We did not observe associations for the majority of age at menopause GWAS signals with length of menstrual cycle, including the four signals with effects of over one-third of a year per allele on menopause timing, implying that the association is specific to *FSHB:* Either FSH-β has independent effects on both cycle length and menopause, or changes in cycle length are causally influencing menopause timing.

Our results are consistent with the observed epidemiological relationship between longer menstrual cycles and later age at menopause [29,30]. It is possible that there is a biological limit on the lifetime number of menstrual cycles, hence women with longer cycles would have later menopause. Alternatively, they may have reduced follicle recruitment per cycle, depleting their ovarian reserve more slowly. Women with longer cycles have more waves of folliculogenesis during each cycle [31,32] but may recruit fewer antral follicles per wave. Oocyte loss due to ovulation is unlikely to be driving the relationship, since this contributes much less to overall oocyte depletion than atresia, there is no robust evidence that preventing ovulation by use of the combined oral contraceptive pill influences menopause timing [29,33-42] and both longer and shorter cycles are more likely to be anovulatory [43]. More work is needed to understand the molecular mechanism that explains the association between cycle length and menopause timing.

The FSH-reducing allele was associated with nulliparity, indicating increased female infertility. Although we were unable to distinguish those unable to have children from those not wishing to, nulliparious women will be enriched for both

female and male factor infertility. The FSH-lowering allele has previously been found to be associated with male infertility [11-14], but we found no association with males who had never fathered a child suggesting a female-specific effect, although this may because the phenotype includes males who chose not to have children in addition to infertile males. Using nulliparity as a proxy for infertility is unlikely to generate a false-positive association, but may have reduced our power to detect a true association. The relationship between FSH and fertility over a woman's lifetime may differ from the age-related changes in FSH around menopause. In contrast to our genetic association between lower FSH and infertility, women nearing menopause have higher FSH concentrations, poorer ovarian reserve and decreased fertility [43,44]. FSH is required for follicle development and it is proposed that an FSH threshold is required to achieve ovulation [7,8]. Ovulation increases with increasing FSH in transgenic mice with FSH levels that increase with age independently of follicle depletion [45]. A high baseline level of FSH, determined by genetic variation, may promote ovulation and explain our association with parity.

The FSH-increasing allele increased the risk of endometriosis in our study. Several GWAS of endometriosis have been performed, however none have reported a signal at the 11p14.1 locus and there was no evidence that the genome-wide significant endometriosis variants were associated with cycle length in our study [27,46-49]. Drug treatments for endometriosis aim to prevent ovulation and menstruation, and to stabilise hormone levels, since oestrogens fuel ectopic endometrial growth [50]. The FSH-increasing allele may similarly stimulate abnormal growth of endometrium. Endometriosis is associated with earlier menopause [40,51] and shorter menstrual cycles [50], consistent with our findings. The FSH-increasing variant associated with increased risk of endometriosis was also associated with parity, however endometriosis can cause infertility as a result of endometriotic lesions and chronic pelvic inflammation. Therefore, the association of the *FSHB* polymorphism with infertility appears to be independent of the association with endometriosis.

We found a modest association of the FSH-lowering allele with increased age at menarche, but the published age at menarche GWAS signals were not associated with length of menstrual cycle. The closest GWAS menarche signal to *FSHB* (rs16918636) is 1.13Mb away and is not in LD (r^2=0.001) with the

FSHB promoter polymorphism SNP [28]. Although FSH is important for normal puberty, the role of variation in baseline FSH levels on puberty timing is uncertain.

UK Biobank recruited individuals over 40 years old, and many of the women still cycling will be approaching menopause, however if the association with cycle length was being driven by peri-menopausal changes we would expect all menopause-associated variants to be associated with cycle length. In addition, our sensitivity analysis suggested a stronger effect of the *FSHB* promoter polymorphism in younger women. We were unable to replicate an association between the FSH-lowering allele and increased odds of PCOS [21]. However, we had only a small number of cases (n=153) limiting our power to detect this association. Other illnesses had relatively small sample sizes and we may have been similarly under-powered. We might have also under-ascertained cases, as most illnesses will be subject to recall bias as they are self-reported and collected retrospectively, while controls might include people not reporting an illness.

Our study provides evidence that a likely functional variant in the *FSHB* promoter is strongly associated with longer menstrual cycles, and to a lesser extent with female infertility and lower risk of endometriosis. There is considerable evidence that the T allele of the *FSHB* promoter polymorphism decreases FSH levels [9-14,16,17], but it has also been associated with increased LH levels [17,21]. While we cannot rule out that the variant may be having direct or indirect effects on other hormone levels, a change in FSH is the most likely primary mechanism. In conclusion, we suggest that lower FSH levels result in longer menstrual cycles and as a consequence later menopause and, while having detrimental effects on female fertility, are protective against endometriosis.

Authors' Roles

A.M. and K.S.R. designed the study, carried out analysis and drafted the article. All authors were involved in designing and performing analysis of the UK Biobank data, revising and approving the manuscript.

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Conflicts of Interest

None to declare.

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Supplementary Methods

Definitions of the reproductive traits used in the analysis

Reproductive trait	Definition
Age at first birth (years)	Age at first live birth (females only).
Age at last birth (years)	Age at last live birth (females only).
Age at menarche (years)	Age at menarche between 9 and 17 years.
Age at natural menopause (years)	Age at last menstrual period excluding those with surgical menopause or taking hormone replacement therapy.
Bilateral oophorectomy	Ever had bilateral oophorectomy (case) vs. never (control).
Breast cancer	Breast cancer on registry (ICD10 C50, ICD9 174&175), vs no cancer reported (control).
Dysmenorrhea	Dysmenorrhea listed as an illness (reported at interview).
Early menarche	Youngest 5% of BMI adjusted age at menarche(case) vs oldest 5% (control). Age at menarche defined as above.
Early menopause	Age at natural menopause (as defined above) at 20-45 years (case) vs. 50-60 years (control).
Endometrial cancer	Endometrial cancer on registry (ICD10 C54, ICD9 182) vs no cancer reported.
Endometriosis	Endometriosis listed as an illness (reported at interview).
Fibroids	Fibroids listed as an illness (reported at interview).
Hysterectomy	Ever had hysterectomy (case) vs. never (control).
Irregular menstrual cycles	Women still menstruating reporting irregular cycles (case) vs. regular cycles (control).
Length of menstrual cycle (days)	Women still menstruating reporting regular cycles. Excludes women taking oral contraceptives, HRT or hormone medications (see list below) and pregnant women.
Long menstrual cycle (vs average)	Length of menstrual cycle >31 days (case) vs 28 days (control). As defined above.
Menopausal symptoms	Menopausal symptoms listed as an illness (reported at interview).
Menorrhagia	Menorrhagia listed as an illness (reported at interview).
Multiple pregnancy loss	Cases are women with two or more pregnancies lost due to still births and miscarriages. Controls are women who have had a live birth and have never had a stillbirth or miscarriage.
Never fathered child	Never fathered a child (case) vs. one or more children fathered.
Never pregnant	Never pregnant (case) vs. one or more pregnancies (control). Number of pregnancies calculated from total number live births, still births, miscarriages, terminations.
Number of children fathered	Number of children fathered. Males only.
Number of live births	Number of live births. Females only.
Ovarian cancer	Ovary cancer on registry (ICD10 C56, ICD9 183) (case) vs no reported cancer (control).
Ovarian cysts	Ovarian cysts listed as an illness (reported at interview).
Polycystic ovary syndrome (PCOS)	Polycystic ovary syndrome listed as an illness (reported at interview).
Short menstrual cycle (vs average)	Length of menstrual cycle ≤20 days (case) vs 28 days (control). As defined above.
Uterine polyps	Uterine polyps listed as an illness (reported at interview).
Vaginal/uterine prolapse	Vaginal/uterine prolapse listed as an illness (reported at interview).

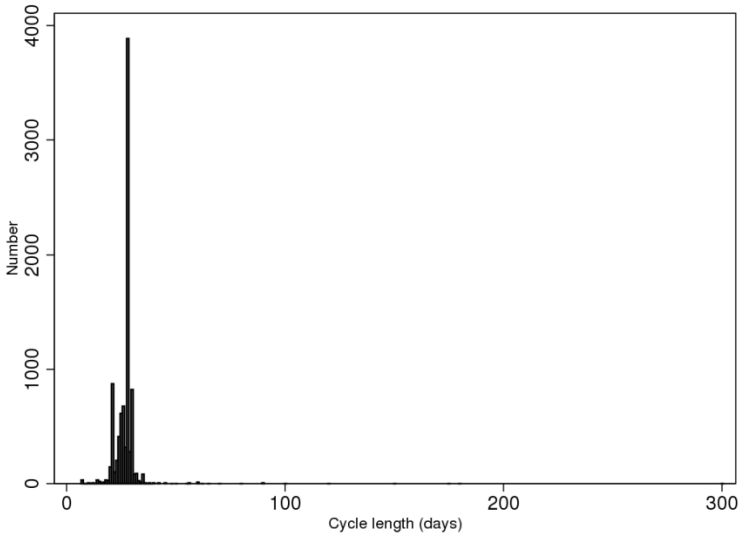
Medications that resulted in exclusion from the length of menstrual cycle analyses:

UK Biobank medication code	UK Biobank description
1141182800	cerazette 75micrograms tablet
1140869346	cilest tablet
1140864196	climagest 1mg tablet
1141172714	climanor 5mg tablet
1140868372	climaval 1mg tablet
1140926430	climesse tablet
1141180944	clomifene
1140884638	clomiphene
1140868406	conjugated oestrogens
1141190580	conjugated oestrogens 0.3mg / medroxyprogesterone 1.5mg tab
1141152356	cyclogest 200mg pessary
1140868590	cyclogest 200mg suppository
1140868508	cyclo-progynova 1mg tablet
1140884634	cyproterone
1141192344	cyproterone acetate+ethinylestradiol
1140876638	cyproterone acetate+ethinyloestradiol
1140868968	danazol
1141157298	danazol product
1140857620	depo-provera 50mg/1ml injection
1140857912	desogestrel
1141182794	desogestrel product
1140880234	dianette tablet
1141156644	elleste duet conti tablet
1140923852	elleste-solo 1mg tablet
1141152228	elleste-solo mx 40 patch
1140926592	estraderm mx 25 patch
1141181700	estradiol product
1141181818	estradiol+norethisterone acetate 1mg/0.5mg tablet
1141202030	estradot 25micrograms patch
1140868470	estrapak 50micrograms/1mg patch+tablet
1140911708	estring 2mg(7.5micrograms/24hours) vaginal ring
1141181594	estriol product
1141181220	ethinylestradiol
1141181218	ethinylestradiol product
1141181286	ethinylestradiol+desogestrel 20mcg/150mcg tablet
1141179822	ethinylestradiol+drospirenone 30micrograms/3mg tablet
1141181306	ethinylestradiol+gestodene 20micrograms/75micrograms tablet
1141181240	ethinylestradiol+levonorgestrel 30mcg/150mcg tablet
1141192874	ethinylestradiol+norelgestromin 600mcg/6mg transdermal patch
1141181298	ethinylestradiol+norethisterone acetate 20mcg/1mg tablet
1141181204	ethinylestradiol+norgestimate 35mcg/250mcg tablet
1140910674	ethinylnortestosterone
1140868446	ethinyloestradiol
1141157404	ethinyloestradiol product
1140869166	ethinyloestradiol+desogestrel 20mcg/150mcg tablet
1140869172	ethinyloestradiol+ethynodiol diacetate 30mcg/2mg tablet
1141166366	ethinyloestradiol+gestodene 20micrograms/75micrograms tablet
1140869248	ethinyloestradiol+levonorgestrel 30mcg/150mcg tablet
1140869328	ethinyloestradiol+norethisterone acetate 20mcg/1mg tablet
1140869348	ethinyloestradiol+norgestimate 35mcg/250mcg tablet
1141166196	etonogestrel
1140916790	evorel 25 patch
1141151718	evorel conti patch
1141192876	evra transdermal patch
1141145900	femara 2.5mg tablet
1140923598	fematrix 40 patch
1140869334	femodene tablet
1141166368	femodette tablet
1140922562	femoston 1/10 tablet
1140923738	femseven 50 patch
1141193320	femtab 1mg tablet

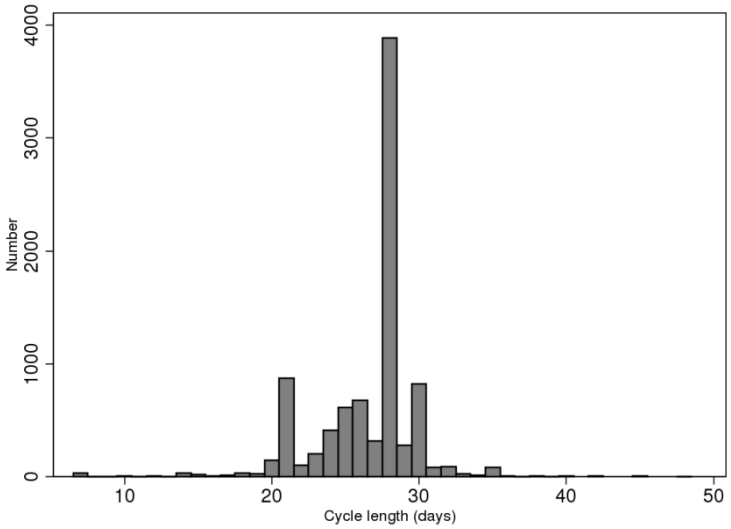
UK Biobank medication code	UK Biobank description
1141193318	femtab continuous tablet
1141193316	femtab sequi tablet
1140869362	femulen tablet
1140868882	gonadorelin
1140909920	gonadotrophin-releasing hormone product
1140870194	goserelin
1141157394	goserelin product
1141184648	human luteinising hormone product
1140882962	human menopausal gonadotrophins
1141166200	implanon 68mg subdermal implant
1141172722	levonelle 750micrograms tablet
1140869366	levonorgestrel
1141157410	levonorgestrel product
1140869162	marvelon tablet
1140858324	medroxyprogest 80mg/ml suspension 100ml
1140869270	medroxyprogesterone
1141177658	menopur 75iu injection (pdr for recon)+solvent
1140884626	mestranol
1141157492	mestranol product
1140869356	mestranol+norethisterone 50micrograms/1mg tablet
1140869180	microgynon 30 tablet
1140869276	micronor tablet
1140869112	mifepristone
1141157302	mifepristone product
1140921822	mirena 20mcg/24hrs intrauterine system
1140921814	mirena 52mg intrauterine system
1140868580	norethisterone
1141157406	norethisterone product
1140869370	norgeston tablet
1140869278	noriday tablet
1140869260	norimin tablet
1140917448	oestradiol 1.25g/dose gel
1140857700	oestradiol 1mg/1ml injection
1140857690	oestradiol 25mg implant 36 week
1140868456	oestradiol product
1141168324	oestradiol+norethisterone acetate 1mg/0.5mg tablet
1140870186	oestrifen 10mg tablet
1140857706	oestriol 250micrograms tablet
1140868400	oestriol product
1141167206	oestrogel 0.06% gel
1140917450	oestrogel 1.25g gel
1140884622	oestrogen product
1140869186	ovranette tablet
1140869262	ovysmen tablet
1140868408	premarin 625micrograms tablet
1140922804	premique 0.625mg/5mg tablet
1140922806	premique cycle 10mg tablet
1140857636	prempak 0.625 tablet
1140868588	progesterone product
1140868460	progynova 1mg tablet
1141180580	progynova ts 50 50micrograms patch
1140923914	progynova ts 50micrograms patch
1140870284	prostap sr 3.75mg injection (pdr for recon)+diluent+kit
1140869190	trinordiol tablet
1140869266	trinovum tablet
1140868514	trisequens tablet
1141179824	yasmin tablet

Supplementary Figures

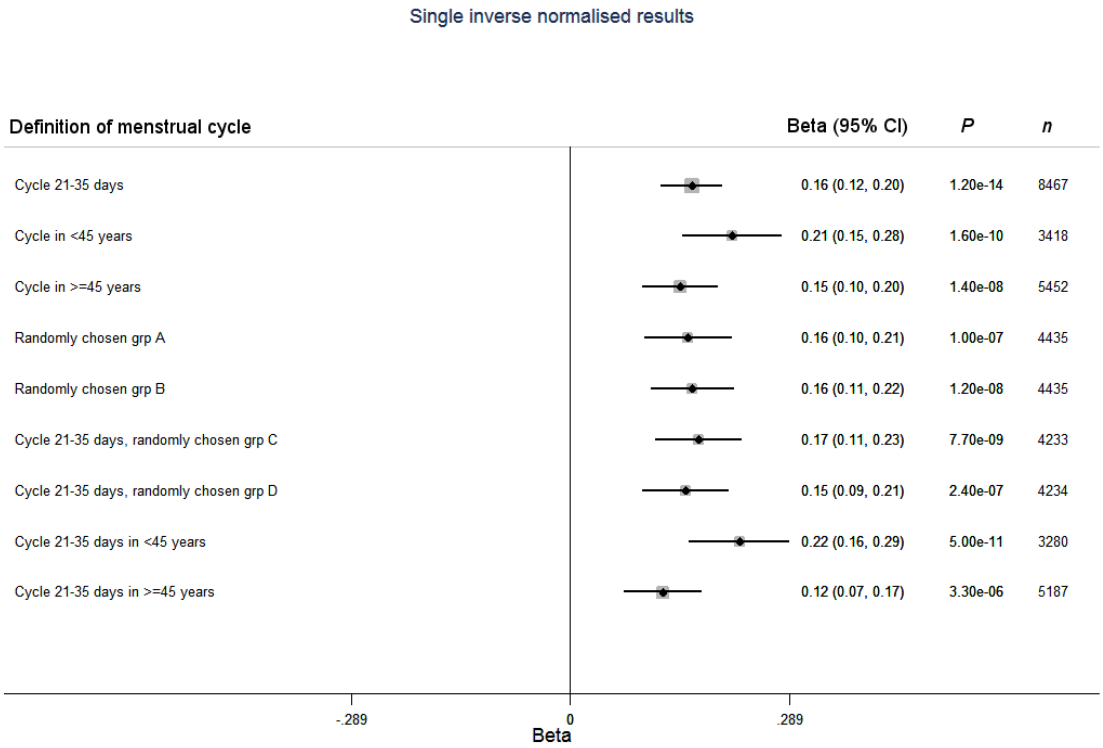
Supplementary Figure 1. Length of menstrual cycle (all).



Supplementary Figure 2. Length of menstrual cycle (cycle length under 50 days).



Supplementary Figure 3. Results of sensitivity analyses for length of menstrual cycle.



	N	Min	Max	Mean	S.D.	Lower quartile	Median	Upper quartile
Cycle 21-35 days	8,467	21	35	26.9	2.8	25	28	28
Cycle in <45 yr	3,418	7	175	26.7	4.7	25	28	28
Cycle >=45 yr	5,452	7	300	26.9	6.9	25	28	28
Randomly chosen grp A	4,435	7	175	26.8	5.4	25	28	28
Randomly chosen grp B	4,435	7	300	26.9	6.9	25	28	28
Cycle 21-35 days, randomly chosen grp C	4,233	21	35	26.8	2.9	25	28	28
Cycle 21-35 days, randomly chosen grp D	4,234	21	35	26.9	2.7	25	28	28
Cycle 21-35 days in <45 years	3,280	21	35	26.9	2.8	25	28	28
Cycle 21-35 days in >=45 years	5,187	21	35	26.9	2.8	25	28	28

Notes: Sensitivity analyses were carried out by restricting the analysis to women with menstrual cycles from 21–35 days; conducting the analysis in women aged under 45 years or 45 years and older; and carrying out the analysis in a split sample of two groups of equal size randomly selected from the full cohort.

Supplementary Table 1. Age at recruitment and cycle length for women included in analysis of length of menstrual cycle.

Phenotype	Analysis	N	Min	Max	Mean	S.D.	Lower quartile	Median	Upper quartile
Length of menstrual cycle (days)	FSHB promoter polymorphism analysis	8,870	7	300	26.8	6.2	25	28	28
Length of menstrual cycle (days)	GWAS	9,534	7	300	26.8	6.1	25	28	28
Age at recruitment (years)	FSHB promoter polymorphism analysis	8,870	40	68	45.9	3.7	43	46	48
Age at recruitment (years)	GWAS	9,534	40	68	45.9	3.7	43	46	48

Notes: The GWAS analysis included genetically related individuals excluded from the analysis of the association of the FSHB promoter polymorphism resulting in an increased total *N*.

Chapter 5:

No evidence of an association between normal length *FMR1* alleles and age at menopause in the general population

Katherine S. Ruth, Claire E. Bennett, Minouk J. Schoemaker,
Michael N. Weedon, Anthony J. Swerdlow, Anna Murray*

Main text

Abstract

Study question: Is the length of *FMR1* repeat alleles in the normal range associated with the clinically-relevant outcome early menopause?

Summary answer: We found no association between normal length *FMR1* repeat alleles and early menopause.

What is known already: There is a non-linear relationship between length of premutation *FMR1* alleles and age at menopause, suggesting that this relationship could continue into the normal range. Within the normal range there is conflicting evidence: differences in ovarian reserve have been identified with *FMR1* repeat allele length, but a recent population-based study of over 3,000 women did not find any association with age at menopause.

Study design, size, duration: Cross-sectional baseline survey data at recruitment during 2004–10 from a population-based, prospective epidemiological cohort study to investigate the causes of breast cancer.

Participants/materials, setting, methods: We included 4,333 women from the Breakthrough Generations Study, of whom 2,118 were early menopause cases (menopause under 46 years) and 2,215 were controls. We analysed the relationship between length of *FMR1* alleles and early menopause using logistic regression with allele length as continuous and categorical variables. We also conducted analyses with the outcome age at menopause as a quantitative trait plus appropriate sensitivity and exploratory analyses.

Main results and the role of chance: There was no association of the shorter or longer *FMR1* allele or their combined genotype with the clinically-relevant endpoint of early menopause ($P>0.01$ for all). Likewise, there were no associations with age at menopause as a quantitative trait ($P>0.05$ for all).

Limitations, reasons for caution: Women with homozygous alleles in the normal range may have undetected *FMR1* premutation alleles, though there was no evidence to suggest this. We estimate minor dilution of risk of early menopause from the inclusion of women with menopause at over 45 years in the early menopause cases due to rounding bias.

Wider implications of the findings: This finding contradicts other smaller, less well-powered studies that suggested differences in primary ovarian insufficiency or ovarian reserve. In addition, we replicate the findings of a similarly-sized study that showed that repeat alleles in the normal range do not affect age at menopause. There is no evidence that normal length *FMR1* repeat alleles influence timing of menopause in the general population.

Introduction

Previous studies have suggested that normal variation in number of CGG repeats in *FMR1* could influence age at menopause. The 5' untranslated region of the *FMR1* gene contains a CGG repeat that varies in length, causing Fragile X syndrome at over 200 repeats, with methylation and silencing of the *FMR1* gene and lack of FMRP expression. While ovarian function remains normal in women with full mutation range repeat alleles, primary ovarian insufficiency (POI) occurs in 20% of women with alleles in the premutation range of *FMR1* (55–200 repeats)[1].

In the premutation range, FMRP is expressed though there are elevated levels of *FMR1* mRNA which has been found to sequester mRNA binding proteins. Although the mechanism of causation for POI remains unknown, premutation range alleles impair follicle development and induce apoptosis in mouse models [2]. Also, there is a non-linear relationship between length of premutation alleles and age at menopause, with earliest menopause at around 80 copies, and later menopause at lower and higher copy numbers [3-5]. It has been hypothesised that this observed relationship with age at menopause may continue to be observed in the range for normal length alleles (<55 repeats).

In white Europeans, the lowest observed allele length is six CGGs, and there are modes in the normal distribution at 20, 30 and 40 CGGs, with 30 repeats being the most common. Previous studies have defined sub-groups based on allele length and have reported differences in ovarian reserve between these [6-8]. In some studies, a greater proportion of alleles of length 35–54 copies have been found in women with POI or diminished ovarian reserve [9-11]. Other studies have not found an association between *FMR1* alleles of length 35–54 copies and POI[12,13] and no relationship of normal length *FMR1* alleles with age at menopause[14]. We tested the role of normal sized *FMR1* CGG repeat alleles in

menopause timing in a cohort of over 2,000 early menopause cases drawn from a population-based study of over 100,000 women from the Breakthrough Generations Study.

Methods

Participants included

In this analysis we included 4,333 women from the Breakthrough Generations Study (BGS) recruited from 2004–10, of whom 2,118 were early menopause cases and 2,215 were controls. BGS is a prospective epidemiological cohort study launched in September 2004 to investigate the environmental, behavioural, hormonal and genetic causes of breast cancer [15] (http://www.breakthroughgenerations.org.uk/). The BGS cohort includes over 110,000 UK women aged 16 and older at entry, recruited through connections to the charity Breakthrough Breast Cancer, volunteers through publicity, and friends, and family members of these. Detailed menstrual histories, and blood samples, have been collected.

Definition of age at natural menopause

Natural menopause was defined as cessation of menstruation for at least 6 months without known cause. We excluded women if periods stopped because of pregnancy, breastfeeding, surgery, hormonal contraceptive use and other types of medical treatment or if there was a medical condition or illness that could have caused amenorrhoea (e.g. polycystic ovary syndrome).

Early menopause cases and controls

Early menopause cases were women with a natural menopause at age 45 years or younger, while controls were women known to be pre-menopausal or to have had menopause (natural or surgical) at 46 years or older. Where possible, controls were selected for each case by matching for date of birth (within 12 months), ethnicity, year of questionnaire completion and source of recruitment. For cases aged under 46 years, a control aged 46 years was selected. When multiple individuals from one pedigree met these criteria, the youngest was included.

Evaluation of FMR1 repeat length

For each subject, Asuragen Amplidex kits (http://www.asuragen. com/) containing *FMR1* CGG repeat region–specific primers were used to PCR amplify the *FMR1* repeat region from 20 ng of genomic DNA that had been extracted from peripheral blood mononuclear cells. All PCRs were performed in 3 μl of reaction volumes in 384-well microtiter plates, using conditions recommended by the kit manufacturers. Products were size separated by capillary electrophoresis on an ABI 3730 automated sequencer (Applied Biosystems, Warrington, UK), using ROX 1000 size standard (Asuragen, Austin, TX, USA) for estimation of product sizes. CGG repeat numbers were determined by comparison with a control individual of 52 CGG repeats. We included duplicates of ~10% of the samples on independent plates. The concordance between duplicate samples was 98.5%, excluding differences of ±1 CGG repeat. Controls included 12 no-template controls, 3 samples from females of known expansion size (largest CGG = 55, 117, and 145), and a lane containing the multiple size targets supplied by Asuragen (CGG = 20, 29, 31, 53, 117, and 196) per 384 plate.

Statistical analyses

We analysed the effect of *FMR1* alleles and genotypes on odds of early menopause using logistic regression in 2,215 early menopause cases and 2,118 controls, and using conditional logistic regression in a set of 1,560 matched case/control pairs. All women had both *FMR1* repeat alleles in the clinically normal range (<55 copies). All statistical analyses were performed using Stata 13.1.

In each woman, we defined ‘Allele 1’ as the shorter *FMR1* repeat allele and ‘Allele 2’ as the longer of her two alleles. We tested the per repeat effect of *FMR1* alleles and also grouped alleles into categories based on size, using previously published criteria [16]: alleles <26 repeats were considered to be ‘low’, 26–34 repeats to be ‘medium’ and >34 repeats to be ‘high’. Statistical analyses were performed for Allele 1 and Allele 2 separately with allele length treated in three ways: as a continuous variable, as a nominal categorical variable (comparison of low and high to medium length as the reference category) or as

an ordinal categorical variable (ordered from low to high). We also repeated the analyses including both alleles from each individual in each of the three models.

In addition to testing the effect of individual alleles we analysed *FMR1* repeat genotypes , with and without an interaction term, or by using the repeat categories to generate six genotypes: low/low, low/medium, low/high, medium/medium, medium/high, high/high. The genotypes were treated as either nominal variables (comparing each genotype to medium/medium) or as ordinal variables, defining the order in two ways: (i) 1. low/low, 2. low/medium, 3. low/high, 4. medium/medium, 5. medium/high, 6. high/high; (ii) 1. low/low, 2.low/medium, 3.medium/medium, 4.low/high, 5.medium/high, 6.high/high. Exploratory analyses were performed of alternative methods of modelling combinations of the alleles: as a difference between allele length in an individual, or as a mean allele length.

In a secondary analysis, we investigated the relationship between *FMR1* alleles and genotypes with age at natural menopause in the 3,805 post-menopausal women in our study. Menopause age was not normally distributed and we therefore ranked values by inverse-normal transformation, to determine the effect of repeat size on menopause as a quantitative trait using linear regression.

Sensitivity analyses were carried out by restricting the analysis to women of white ethnicity and including smoking status at time of study entry as a covariate. Smoking status and ethnicity were available for 4,017 of the women analysed (Table 1). In the analysis of menopause as a quantitative trait, we also tested the models separately in the control group.

Statistical power was estimated using Quanto (http://biostats.usc.edu/Quanto.html). We estimated the size of genetic effect we could detect at 80% power for an additive mode of inheritance with the low/low genotype as ‘aa’, low/medium as ‘aA’ and medium/medium as ‘AA’, with sample size and allele frequency estimated from the number of women with these genotypes. For case–control models, we assumed a population prevalence of 5% for early menopause.

Table 1. Ethnicity and smoking status of women included in the study (available for 4,017 women analysed).

Genotype	Women with natural menopause		Early menopause cases		Early menopause controls	
	n	%	n	%	n	%
White ethnicity	3,484	99.5	2,099	99.5	1,896	99.4
Non-white	18	0.5	11	0.5	11	0.6
Never smoker	2,132	60.9	1,239	58.7	1,228	64.4
Former smoker	1,125	32.1	680	32.2	593	31.1
Current smoker	239	6.8	185	8.8	86	4.5
Smoking status not known	6	0.2	6	0.3	0	0.0
Total	**3,502**		**2,110**		**1,907**	

Results

Repeat distribution

The length of the *FMR1* allele repeat ranged from 7 to 54 copies (Figure 1). The distribution of the *FMR1* allele was consistent with previous studies with a mode at 30 copies, and secondary peaks at 20 and 23 copies [17,18], with similar distributions in the early menopause cases and the rest of the cohort. Almost half of women with a natural age at menopause had two medium length alleles (26–34 repeats), and almost one-third had a combination of one low allele (<26 repeats) and one medium allele (26–34 repeats) (Table 2).

Age of menopause

In early menopause cases (n=2,118), the median age at natural menopause was 43 years (range 19–45 years, mean 42.4 years, s.d. 3.1 years) compared with 52 years in the controls (n=1,687, range 46–62 years, mean 52.1 years, s.d. 3.1 years), although 24% (n=528) of controls were pre-menopausal and therefore could not be included in the mean. Overall, the median age at natural menopause was 45 years in cases and controls combined (n=3,805, range 19–62 years, mean 46.7 years, s.d. 5.7 years), which was lower than the median population menopause age of 50 years due to the large proportion of early menopause cases (56%).

Figure 1. FMR1 allele length in early menopause cases and controls. The percentage of alleles at each length is in early menopause cases (n=4,236) and controls (n=4,430).

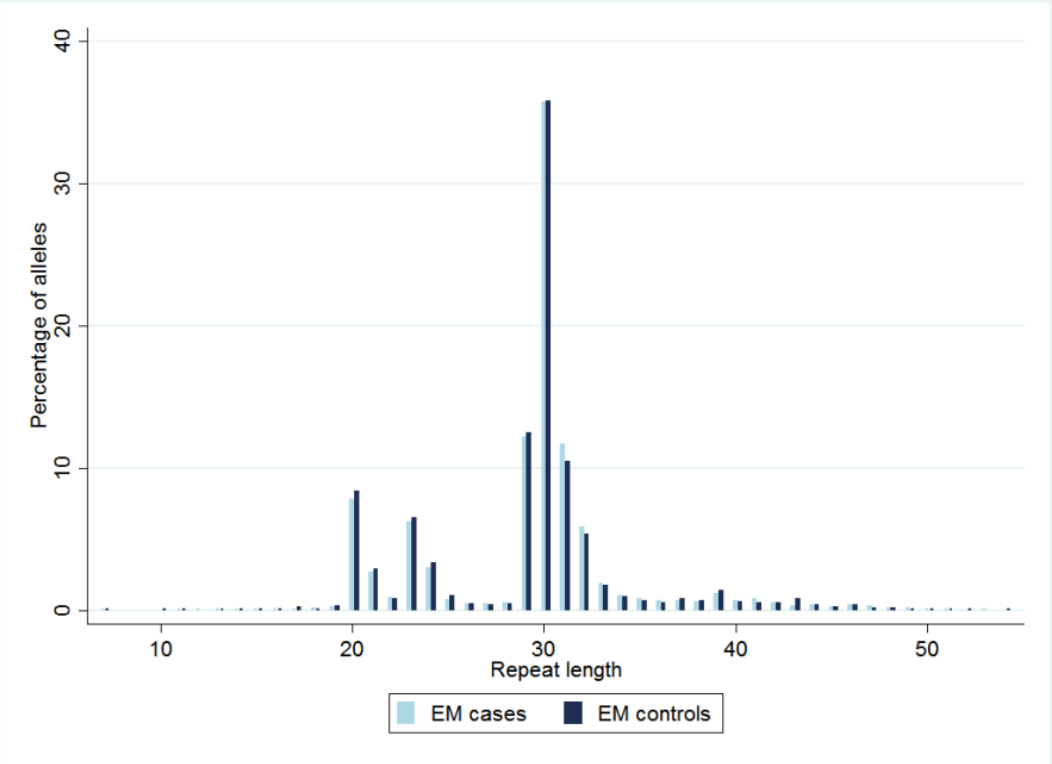


Table 2. Number of women by FMR1 genotype, categorised by allele lengths.

Genotype	Post-menopausal women included in quantitative trait analysis		Early menopause cases		Early menopause controls	
	n	%	n	%	n	%
Low/low	204	5.4	130	5.9	101	4.8
Low/medium	1,215	31.9	710	32.1	662	31.3
Low/high	145	3.8	88	4.0	77	3.6
Medium/medium	1,803	47.4	1,027	46.4	1,035	48.9
Medium/high	409	10.7	248	11.2	221	10.4
High/high	29	0.8	12	0.5	22	1.0
Total	**3,805**	**100.0**	**2,215**	**100.0**	**2,118**	**100.0**

Note: Low<26 repeats; medium 26–34 repeats; high 35–54 repeats.

No association between length of either FMR1 repeat allele and early menopause

We found no associations of either *FMR1* allele with odds of early menopause (Table 3), other than a nominal association of 'low' Allele 2 with decreased odds of early menopause in the matched case–control analysis (OR=0.72, 95% CI 0.52–0.99, *P*=0.044) (*P*>0.05 for all other results). There was no association between length of either Allele 1 or Allele 2 of *FMR1* and age at menopause as a quantitative trait (*P*>0.05 for all). When the analysis was carried out by including both alleles (including each outcome twice in the data, once for each chromosome carrying the repeat allele) there was no association between *FMR1* allele length and odds of early menopause or age at menopause (*P*>0.05 for all).

No association between FMR1 repeat genotype and early menopause

FMR1 repeat genotype was not associated with early menopause (Table 4), except for a nominal association of the 'low/low' genotype with reduced odds of early menopause in the matched case–control analysis (OR=0.69; 95% CI 0.50–0.96; *P*=0.027) (*P*>0.05 for all other results). There were no associations between *FMR1* repeat genotype and age at menopause as a quantitative trait (*P*>0.05 for all).

Sensitivity analyses

Although we identified smoking as a potential confounder, smoking was not associated with length of *FMR1* allele or genotype (p>0.05 for all), and when analyses were adjusted for smoking, results were consistent with the main analysis. Results remained consistent when analyses were restricted to women of white ethnicity or when the secondary analysis of age at menopause was carried out in only the control group.

Table 3. Relationship of FMR1 allele length with early menopause and age at menopause as a quantitative trait.

Model	Variables included	Early menopause (n=2,215 cases, n=2,118 controls)				Age at natural menopause (n=3,805)			
		OR	**95% CI**	**SE**	***P***	**Beta**	**95% CI**	**SE**	***P***
Allele 1, continuous	Allele 1 (cont.)	1.012	(0.999,1.026)	0.007	0.077	-0.004	(-0.011,0.003)	0.004	0.280
Allele 1, nominal categorical	1. low	0.919	(0.814,1.038)	0.057	0.173	0.051	(-0.014,0.115)	0.033	0.125
	2. medium (ref.)	Ref.				Ref.			
	3. high	1.861	(0.917,3.777)	0.672	0.085	-0.263	(-0.628,0.103)	0.187	0.159
Allele 1, ordinal categorical	1. low, 2. medium, 3.high	1.113	(0.989,1.252)	0.067	0.075	-0.059	(-0.122,0.003)	0.032	0.063
Allele 2, continuous	Allele 2 (cont.)	1.002	(0.989,1.015)	0.007	0.750	-0.001	(-0.008,0.005)	0.003	0.672
Allele 2, nominal categorical	1. low	0.795	(0.608,1.04)	0.109	0.094	0.049	(-0.067,0.216)	0.075	0.302
	2. medium (ref.)	Ref.				Ref.			
	3. high	0.941	(0.797,1.111)	0.080	0.474	0.009	(-0.07,0.107)	0.019	0.679
Allele 2, ordinal categorical	1. low, 2. medium, 3.high	1.026	(0.897,1.173)	0.070	0.712	-0.009	(-0.081,0.062)	0.036	0.800

ref.=reference; cont.=continuous

Table 4. Relationship of FMR1 genotype with early menopause and age at menopause as a quantitative trait.

Model	Variables included	Early menopause (n=2,215 cases, n=2,118 controls)				Age at natural menopause (n=3,805)			
		Beta	**95% CI**	**SE**	***P***	**Beta**	**95% CI**	**SE**	***P***
Allele 1, Allele 2 and interaction, continuous	Allele 1 (cont.)	0.978	(0.904,1.059)	0.039	0.586	0.002	(-0.041,0.044)	0.022	0.934
	Allele 2 (cont.)	0.970	(0.908,1.036)	0.033	0.367	0.004	(-0.032,0.039)	0.018	0.836
	Interaction	1.001	(0.999,1.004)	0.001	0.384	0.000	(-0.001,0.001)	0.001	0.793
Genotype, nominal categorical	1. low/ low	0.771	(0.586,1.014)	0.108	0.063	0.094	(-0.051,0.239)	0.074	0.202
	2. low/ medium	0.925	(0.807,1.061)	0.064	0.265	0.057	(-0.016,0.129)	0.037	0.127
	3. low/ high	0.868	(0.632,1.193)	0.141	0.384	0.043	(-0.126,0.212)	0.086	0.619
	4. medium/ medium (ref.)	Ref.				Ref.			
	5. medium/ high	0.884	(0.723,1.081)	0.091	0.230	0.051	(-0.056,0.158)	0.055	0.349
	6. high/ high	1.819	(0.896,3.695)	0.658	0.098	-0.245	(-0.611,0.121)	0.187	0.190
Genotype, ordinal categorical (order 1)	1. low/ low 2. low/ medium 3. low/ high 4. medium/ medium 5. medium/ high 6. high/ high	1.039	(0.989,1.092)	0.026	0.132	-0.022	(-0.048,0.005)	0.014	0.110
Genotype, ordinal categorical (order 2)	1. low/ low 2.low/ medium 3.medium/ medium 4.low/ high 5.medium/ high 6.high/ high	1.025	(0.967,1.087)	0.030	0.399	-0.016	(-0.047,0.015)	0.016	0.309

ref.=reference. cont.=continuous. Notes: The genotypes were treated as either nominal variables (comparing each genotype to medium/medium) or as ordinal variables, defining the order in two ways: (i) 1. low/low, 2. low/medium, 3. low/high, 4. medium/medium, 5. medium/high, 6. high/high; (ii) 1. low/low, 2.low/medium, 3.medium/medium, 4.low/high, 5.medium/high, 6.high/high.

Discussion

We found no association between normal length *FMR1* alleles and early menopause. This unlikely to be due to a lack of power since we estimated that we were powered to detect an odds ratio <0.85 or >1.18 per low allele in the unmatched case–control analysis (similar values for matched analysis). This is similar in size to effect estimates of 1.13 to 1.85 per allele for common single nucleotide polymorphisms (SNPs) in the same study cohort [19]. For the analysis of age at menopause as a quantitative trait, we estimated that we were powered to detect a change of about 0.5 years per low allele, similar to the 0.1–0.9 years per allele effect sizes for common SNPs [20]. Indeed we were able to detect a strong effect of smoking on reducing age of menopause, a well-characterised environmental risk factor for earlier menopause [21]. As well as demonstrating no association between normal *FMR1* allele length and risk of early menopause, our results corroborate a null association between *FMR1* normal length alleles and quantitative age at menopause from a population-based study [14].

From the matched case–control analysis there was a suggestion that having *FMR1* repeats <26 CGGs was associated with lower odds of earlier menopause. However, this association is only nominally significant and would not pass a more stringent significance threshold of $P<0.01$, based on the five tests carried out comparing each genotype to medium/medium. If an association of the shortest *FMR1* repeats with reduced odds of early menopause were confirmed, this would contradict a study that found accelerated loss of ovarian reserve in women with low alleles and better ovarian reserve with high alleles [16].

Although we were well-powered to detect an association, our calculations do not take into account factors affecting the accuracy of the data collected or that would have reduced our power to detect an association. Long *FMR1* alleles are harder to detect; therefore women with homozygous alleles may actually be heterozygotes with an undetected premutation repeat. Of the 4,333 women, 21% were homozygotes but there was high concordance between duplicated samples, and the proportion of homozygotes in early menopause cases and controls was not statistically different. We would expect such genotyping errors

to result in a higher proportion of homozygotes in early menopause cases, since premutation repeats are a known aetiological risk factor for early menopause [22].

Another factor that might have contributed to the lack of an observed association is the potential dilution of risk of early menopause from misidentification of early menopause cases. Previous studies have observed rounding bias towards reporting values ending in 0 or 5 when women are asked to recall their age at menopause [23], therefore some women may have rounded down their menopause age to 45. We estimate that this may have occurred in 7% of early menopause cases, hence the consequent dilution of risk would have been minor and does not account for the lack of an observed association. We controlled for two potential confounders in our analysis: ethnicity and smoking. Ethnicity is known to affect *FMR1* allele length [18], and we found no association with smoking.

In summary, in a large population based study, we found no association between normal length *FMR1* repeats and risk of the clinically-relevant outcome early menopause, and replicated a null association with age at menopause as a quantitative trait.

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Chapter 6:
Large-scale genomic analyses link reproductive ageing to hypothalamic signaling, breast cancer susceptibility and BRCA1-mediated DNA repair

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Main text

Abstract

Menopause timing has a substantial impact on infertility and risk of disease, including breast cancer, but the underlying mechanisms are poorly understood. We report a dual strategy in ~70,000 women to identify common and low-frequency protein-coding variation associated with age at natural menopause (ANM). We identified 44 regions with common variants, including two harbouring additional rare missense alleles of large effect. We found enrichment of signals in/near genes involved in delayed puberty, highlighting the first molecular links between the onset and end of reproductive lifespan. Pathway analyses revealed a major association with DNA damage-response (DDR) genes, including the first common coding variant in *BRCA1* associated with any complex trait. Mendelian randomisation analyses supported a causal effect of later ANM on breast cancer risk (~6% risk increase per-year, $P=3x10^{-14}$), likely mediated by prolonged sex hormone exposure, rather than DDR mechanisms.

Introduction

Younger age at natural (non-surgical) menopause (ANM) is associated with lower risk of breast cancer, but higher risks of osteoporosis, cardiovascular disease and type 2 diabetes [1]. Early menopause also has a substantial impact on fertility. It is estimated that natural fertility ceases on average 10 years before menopause [2], which is becoming increasingly relevant as women in many populations are delaying childbearing. For example, the birth rate in British women aged 30-34 years is now higher than in any other half decade (http://www.ons.gov.uk/ons/publications/). ANM is on average 51 years in Caucasian populations, while natural menopause before the age of 40, or primary ovarian insufficiency (POI), occurs in 1% of the population [3].

Previous genome wide association studies (GWAS) identified 18 common genetic loci associated with ANM, implicating several plausible gene candidates across a number of molecular pathways [4,5]. Together those reported variants explained <5% of the variation in ANM, compared to 21% explained by all common variants on GWAS arrays [4]. We therefore undertook a more comprehensive genetic analysis in a substantially larger sample of nearly

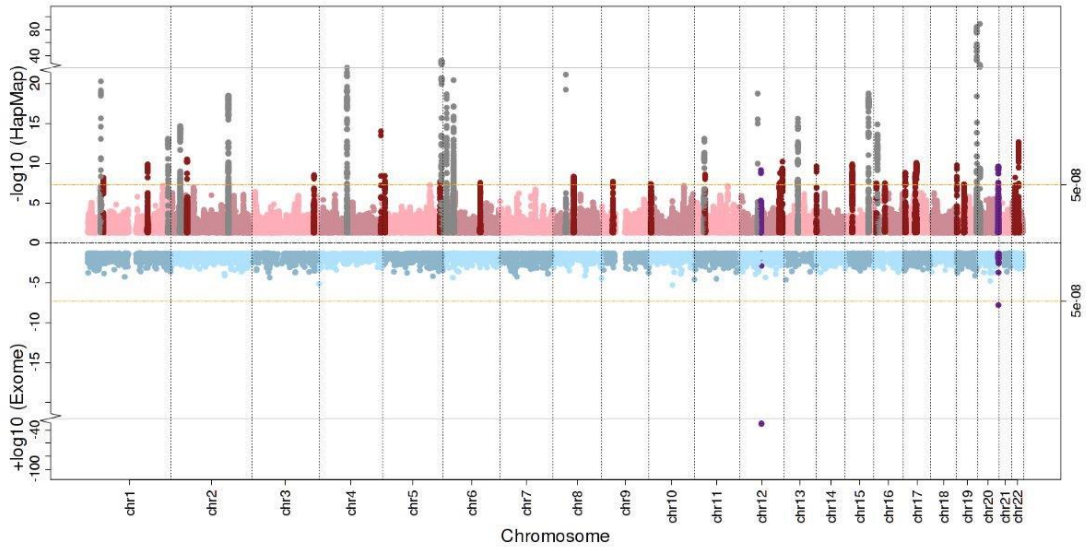
70,000 women, incorporating both common and, for the first time, low-frequency coding variants. We were able to triple the number of independent signals associated with ANM, including two low frequency coding variants in previously unreported loci. Our findings provide new insights into the causal relationship between ANM with breast cancer and identify molecular overlaps between ANM and puberty timing.

Results

GWAS HapMap 2 meta-analysis

In a combined analysis of up to 69,360 women of European ancestry (Supplementary Table 1), 1,208 SNPs, among a total of ~2.6 million, reached the genome-wide significance threshold ($P<5x10^{-8}$) for association with ANM. Of these, we identified 54 independent signals located in 44 genomic regions using approximate conditional analysis implemented in GCTA (Figure 1, Table 1, Supplementary Tables 2 and 3). Eight loci contained secondary signals: six loci each contained two signals, and two loci each contained three signals. Across the 54 identified signals, MAFs ranged from 7% to 49%, and effect sizes from 0.07 to 0.88 years per allele with no significant heterogeneity between studies. All of the 18 previously reported independent signals for ANM [4,5] retained directionally concordant genome-wide significance (maximum $P=3.7x10^{-11}$). These 18 signals were also directionally concordant in a sub-meta-analysis of studies that were not included in the previous publication (P-value range $1x10^{-30}$ to $1x10^{-3}$). The top 29,958 independent SNPs with association $P<0.05$ explained 21% (SE 9.7%, P=0.01) of the variance in ANM reducing to 6% (SE 1.6%, $P=6.3x10^{-12}$) for the top 54 SNPs with $P<5x10^{-8}$ (Supplementary Table 4). This contrasts with an estimate of 2.6% for the previously identified 18 index SNPs.

Figure 1. Miami plot of HapMap and exome SNP associations.



Notes: Log-transformed P values are shown for association with ANM for SNPs from HapMap 2 (top; pink) and SNPs from the meta-analysis of exome chip data (bottom; blue). Previously known signals are shown in gray, and newly discovered signals are shown in red (HapMap 2) or purple (exome chip and HapMap 2). The yellow lines correspond to genome-wide significant levels in each direction; the gray lines indicate where the y axis has been truncated.

Table 1. Association of 54 common HapMap 2 variants at 44 genomic loci with ANM

							Univariate Model[5]		Joint Model[6]		
Region	**Best SNP[1]**	**Signal SNP[2]**	**Chr**	**Position[3]**	**Alleles[4]**	**N**	**Effect**	**P**	**Effect**	**P**	**Highlighted Gene[7]**
1*	rs4246511	rs4246511	1	39,152,972	c/t/0.71	69116	-0.22 (0.02)	5.1E-21	-	-	*RHBDL2*$^{(B,N)}$ / ***MYCBP***$^{(B)}$
2	rs12142240	rs12142240	1	46,519,888	t/c/0.68	69356	-0.13 (0.02)	6.6E-09	-	-	***RAD54L***$^{(B,E)}$
3	rs1411478	rs1411478	1	179,228,905	a/g/0.41	68680	-0.13 (0.02)	1.4E-10	-	-	*STX6*$^{(N,E)}$
4*	rs2236918	rs2236918	1	240,084,449	c/g/0.45	69332	-0.15 (0.02)	8.3E-14	-	-	***EXO1***$^{(N,B,C)}$
5*	rs704795	rs704795	2	27,569,998	a/g/0.4	69341	-0.16 (0.02)	2.1E-15	-	-	***BRE***$^{(B)}$ / *GTF3C2*$^{(B,E)}$ / *EIFB4*$^{(B)}$
6*	rs1800932	rs1800932	2	47,871,585	a/g/0.81	69309	-0.17 (0.03)	3.2E-11	-	-	***MSH6***$^{(N,B,E)}$
7*	rs930036	rs930036	2	171,649,264	a/g/0.38	69357	-0.19 (0.02)	3.1E-19	-	-	***TLK1***$^{(N,E,B)}$ / *GAD1*$^{(B)}$
8	rs16858210	rs16858210	3	185,106,704	g/a/0.75	69193	-0.14 (0.02)	3.1E-09	-	-	***PARL***$^{(B)}$ / ***POLR2H***$^{(B)}$
9*	rs4693089	rs4693089	4	84,592,646	a/g/0.51	69060	-0.20 (0.02)	9.2E-23	-	-	***HELQ***$^{(N,B)}$ / ***FAM175A***$^{(B)}$
10	rs6856693	rs6856693	4	185,985,800	a/g/0.58	67635	-0.16 (0.02)	9.8E-15	-	-	*ASCL1*$^{(N)}$, ***MLF1IP***$^{(B)}$
11	rs427394	rs427394	5	6,798,875	g/a/0.41	69284	-0.13 (0.02)	3.8E-09	-	-	***PAPD7***$^{(N,B)}$
12	rs11738223	rs11738223	5	171,867,097	a/g/0.68	69250	-0.12 (0.02)	2.0E-08	-	-	*SH3PXD2B*$^{(N)}$
13a*	rs365132	rs2241584	5	175,888,783	a/g/0.38	69341	-0.14 (0.02)	1.5E-11	-0.14 (0.02)	3.2E-11	***UIMC1***$^{(B,E)}$
13b*	"	rs365132	5	176,311,180	g/t/0.51	69349	-0.24 (0.02)	1.4E-33	-0.24 (0.02)	7.9E-33	***UIMC1***$^{(N,B,E)}$
14a*	rs6899676	rs6899676	6	11,003,246	a/g/0.8	69303	-0.23 (0.03)	2.2E-19	-0.21 (0.03)	6.2E-16	*SYCP2L*$^{(N,B)}$ / *MAK*$^{(B)}$
14b*	"	rs9393800	6	11,059,723	g/a/0.27	69124	-0.17 (0.02)	3.5E-13	-0.14 (0.02)	1.1E-09	*SYCP2L*$^{(N,B)}$ / *MAK*$^{(B)}$
15a*	rs1046089	rs2230365	6	31,633,427	c/t/0.84	67095	-0.17 (0.03)	7.6E-10	-0.16 (0.03)	2.7E-08	***MSH5***$^{(B)}$ / *HLA*$^{(B)}$
15b*	"	rs707938	6	31,837,338	g/a/0.32	68582	-0.17 (0.02)	7.2E-15	-0.16 (0.02)	2.3E-13	***MSH5***$^{(B,N,E)}$ / *HLA*$^{(B)}$
16	rs12196873	rs12196873	6	111,704,751	a/c/0.85	69313	-0.16 (0.03)	2.8E-08	-	-	***REV3L***$^{(B,C)}$
17*	rs2720044	rs2720044	8	38,099,744	a/c/0.84	63917	-0.29 (0.03)	7.3E-22	-	-	*STAR*$^{(B)}$
18	rs10957156	rs10957156	8	61,791,955	a/g/0.76	69341	-0.14 (0.02)	4.5E-09	-	-	***CHD7***$^{(N,B,E)}$
19	rs4879656	rs4879656	9	33,002,382	a/c/0.37	68919	-0.12 (0.02)	2.0E-08	-	-	***APTX***$^{(N,B,E)}$
20	rs10905065	rs10905065	10	5,809,833	a/g/0.61	69334	-0.11 (0.02)	3.9E-08	-	-	***FBXO18***$^{(B)}$
21a*	rs11031006	rs11031006	11	30,183,104	g/a/0.85	69309	-0.22 (0.03)	8.5E-14	-0.25 (0.03)	4.0E-17	*FSHB*$^{(N,B)}$
21b*	"	rs6484478	11	30,263,016	g/a/0.74	69099	-0.10 (0.02)	4.0E-05	-0.14 (0.02)	1.0E-08	*FSHB*$^{(B)}$
22	rs10734411	rs10734411	11	32,498,360	a/g/0.47	69142	-0.12 (0.02)	2.6E-09	-	-	*EIF3M*$^{(N)}$
23*	rs2277339	rs2277339	12	55,432,336	g/t/0.1	67603	-0.31 (0.03)	1.8E-19	-	-	***PRIM1***$^{(B,N,C,E)}$ / *TAC3*$^{(B)}$
24a	rs12371165	rs3741604	12	64,982,677	t/c/0.52	69100	-0.09 (0.02)	1.9E-05	-0.29 (0.03)	1.8E-21	***HELB***$^{(N,B,E,C)}$
24b	"	rs1183272	12	65,021,688	c/t/0.45	68727	-0.07 (0.02)	7.3E-04	-0.31 (0.03)	3.0E-24	***HELB***$^{(B,N,C)}$
24c	"	rs7397861	12	65,100,733	g/c/0.64	69095	-0.10 (0.02)	6.7E-06	-0.13 (0.02)	4.6E-09	***HELB***$^{(B,E,C)}$
25	rs551087	rs551087	12	119,693,576	g/a/0.29	69001	-0.13 (0.02)	3.9E-08	-	-	*SPPL3*$^{(N)}$ / *SRSF9*$^{(B)}$

							Univariate Model[5]		Joint Model[6]		
Region	**Best SNP[1]**	**Signal SNP[2]**	**Chr**	**Position[3]**	**Alleles[4]**	**N**	**Effect**	**P**	**Effect**	**P**	**Highlighted Gene[7]**
26	rs1727326	rs1727326	12	122,166,039	c/g/0.15	68870	-0.19 (0.03)	1.7E-09	-	-	***KNTC1***$^{(B)}$, *PITPNM2*$^{(N)}$
27	rs12824058	rs12824058	12	129,370,287	g/a/0.43	69047	-0.14 (0.02)	6.1E-11	-	-	*PIWIL1*$^{(N)}$
28*	rs4886238	rs4886238	13	60,011,740	g/a/0.66	69314	-0.18 (0.02)	2.5E-16	-	-	*TDRD3*$^{(B,N)}$
29	rs1713460	rs1713460	14	20,003,455	g/a/0.3	68528	-0.14 (0.02)	2.4E-10	-	-	***APEX1***$^{(B)}$ / ***PARP2***$^{(B)}$ / *PNP*$^{(N,E)}$
30	rs9796	rs9796	15	39,058,739	t/a/0.46	69317	-0.13 (0.02)	1.3E-10	-	-	***INO80***$^{(B,N,E)}$ / ***RAD51***$^{(B)}$
31*	rs1054875	rs1054875	15	87,680,130	t/a/0.4	69288	-0.19 (0.02)	1.7E-19	-	-	***POLG***$^{(B,N)}$ / ***FANCI***$^{(B,C)}$
32	rs9039	rs9039	16	9,112,864	c/t/0.28	69341	-0.12 (0.02)	3.3E-08	-	-	*C16orf72*$^{(N)}$ / *ABAT*$^{(B)}$
33*	rs10852344	rs10852344	16	11,924,420	t/c/0.59	69346	-0.16 (0.02)	1.3E-15	-	-	*GSPT1*$^{(N,C,E)}$ / *BCAR4*$^{(B)}$
34	rs12599106	rs12599106	16	34,355,526	a/t/0.51	69320	-0.12 (0.02)	3.1E-08	-	-	*UBE2MP1*$^{(N)}$
35	rs8070740	rs8070740	17	5,272,620	a/g/0.76	68515	-0.15 (0.02)	1.5E-09	-	-	*RPAIN*$^{(N,E)}$
36	rs2941505	rs2941505	17	35,086,230	a/g/0.32	69302	-0.13 (0.02)	1.9E-09	-	-	*STARD3*$^{(B)}$ / *PGAP3*$^{(N,E)}$ / *CDK12*$^{(B)}$
37	rs1799949	rs1799949	17	38,498,992	g/a/0.68	69329	-0.14 (0.02)	8.4E-11	-	-	***BRCA1***$^{(N,E,B,C)}$
38	rs349306	rs349306	19	901,694	g/a/0.13	58278	-0.23 (0.04)	1.7E-10	-	-	***POLR2E***$^{(B)}$ / *KISS1R*$^{(B)}$
39	rs7259376	rs7259376	19	22,299,545	a/g/0.46	69328	-0.11 (0.02)	4.2E-08	-	-	*ZNF729*$^{(N)}$
40a*	rs11668344	rs11668344	19	60,525,476	g/a/0.36	69329	-0.41 (0.02)	5.5E-85	-0.41 (0.02)	4.2E-84	***BRSK1***$^{(B,E)}$ / *NLRP11*$^{(N)}$ / *U2AF2*$^{(B)}$
40b*	"	rs2547274	19	61,002,040	g/c/0.91	66580	-0.28 (0.04)	3.4E-13	-0.22 (0.04)	2.7E-08	***BRSK1***$^{(B)}$ / *NLRP11*$^{(N)}$ / *U2AF2*$^{(B)}$
40c*	"	rs12461110	19	61,012,475	a/g/0.35	68518	-0.17 (0.02)	7.6E-16	-0.15 (0.02)	5.0E-12	***BRSK1***$^{(B)}$ / *NLRP11*$^{(N,C)}$ / *U2AF2*$^{(B)}$
41a*	rs16991615	rs451417	20	5,889,999	a/c/0.12	65420	-0.20 (0.03)	4.6E-09	-0.2 (0.03)	4.5E-09	***MCM8***$^{(N,C,B)}$
41b*	"	rs16991615	20	5,896,227	g/a/0.93	66210	-0.88 (0.04)	1.6E-89	-0.88 (0.04)	4.4E-89	***MCM8***$^{(N,C,B)}$
42a	rs13040088	rs2236553	20	60,760,188	c/t/0.24	62648	-0.16 (0.03)	6.1E-10	-0.16 (0.03)	4.4E-10	*SLCO4A1*$^{(N,C)}$ / ***DIDO1***$^{(B,E)}$
42b	"	rs13040088	20	61,019,647	g/a/0.21	69317	-0.16 (0.02)	2.4E-10	-0.16 (0.02)	1.9E-10	*SLCO4A1*$^{(C)}$ / ***DIDO1***$^{(N,B,E)}$
43	rs5762534	rs5762534	22	26,963,571	t/c/0.84	69322	-0.16 (0.03)	6.1E-09	-	-	***CHEK2***$^{(B)}$
44	rs763121	rs763121	22	37,209,886	g/a/0.36	66632	-0.16 (0.02)	2.3E-13	-	-	***DMC1***$^{(B)}$ / *DDX17*$^{(N,E,B)}$

1. Best regional SNP selected by 1Mb distance based clumping, 2. Lead independent SNP(s) in region selected through approximate conditional analysis, 3. Position in build 36, 4. Effect allele / other allele / effect allele frequency, 5. Univariate test statistics reported from the primary meta-analysis (i.e no conditional analysis). 6.Test statistics derived from the joint model for regions containing more than one statistically independent SNP, 7. Highlighted gene in region based on following criteria: (N) = Nearest, (B) = Biological Candidate, (E) = eQTL effect, (C) non-synonymous SNP in high LD. Genes categorised as "DDR" are shown in bold. * denotes a region previously described at genome-wide significance.

We assessed functional enrichment of all ANM-SNP associations in regions containing active histone marks across 10 physiological cell-type groups using stratified LD score regression [6] (see Methods and Supplementary Table 5). Only the 'kidney related cell types' group showed significant enrichment (P=0.003), which could reflect the mesonephric embryonic origin of ovarian parenchymal cells [7]. Analysis by functional annotation revealed the strongest enrichment for variants located in UCSC defined coding regions (Supplementary Table 5), with ~1.5% of SNPs explaining 24.8% of the trait heritability ($P=4.6x10^{-3}$). The heritable component increased to 55% (SE 11%, $P=2.9x10^{-7}$) when a 500bp window was added to the coding regions, capturing ~6.5% of SNPs.

Exome array meta-analysis

To estimate the contribution of low-frequency coding variation to ANM, we performed a meta-analysis of up to 39,026 women genotyped on exome arrays (Supplementary Table 6). Only one signal, from two highly correlated ($r^2=0.73$, D'=1) low-frequency missense variants in *HELB*, reached genome-wide significance in this discovery phase (Table 2, Figure 1, Supplementary Table 7). Ten low-frequency (MAF<5%), non-synonymous SNPs with association $P<5x10^{-4}$ were selected for follow-up in an independent sample of 10,157 women from the deCODE study that imputed rare variant genotypes. Directionally concordant effect estimates were observed for 6/8 variants (2 of the 10 failed QC). The combined analysis identified missense alleles in *HELB* (rs75770066, MAF= 3.6%, beta = 0.85 year/allele, $P=1.2x10^{-31}$) and *SLCO4A1* (rs140267842, MAF= 0.8%, beta=0.79, $P=1.6x10^{-8}$) as associated with ANM (Table 2, Supplementary Table 7 and Supplementary Figure 1).

Table 2. Results of the exome chip meta-analyses.

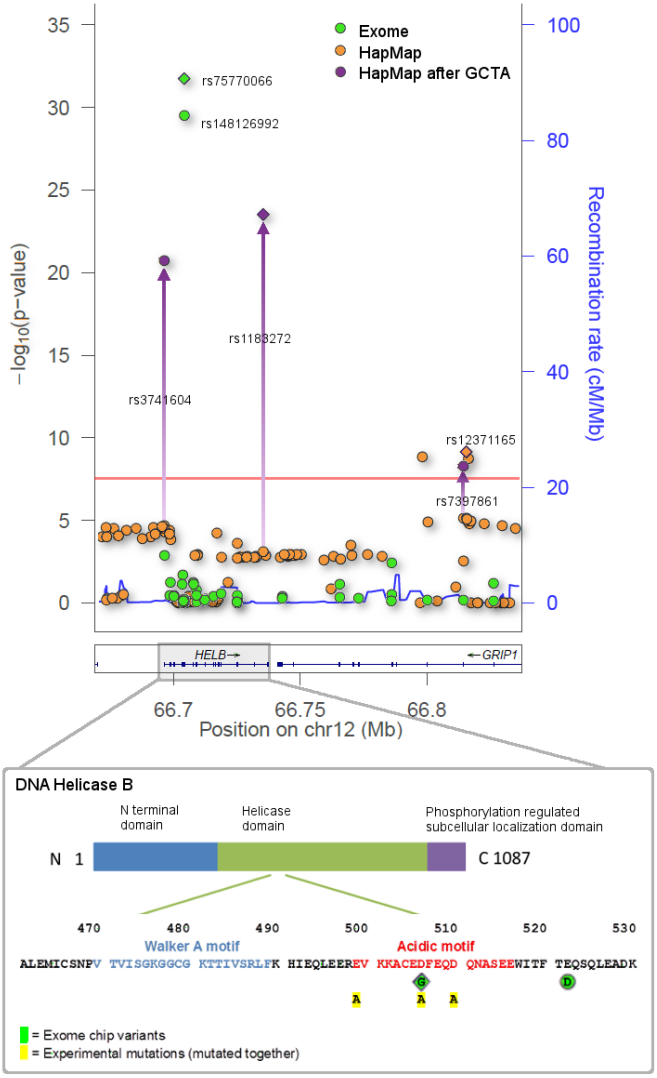
SNP	Band	Gene	Amino acid change	Minor/ common allele	Analysis	MAF (%)	Effect (SE) of minor allele in years	p-value	n	Heterogeneity p-value
rs75770066	12q14.3	*HELB*	p.Asp506Gly	G/A	Discovery	3.6	0.91 (0.08)	**1.79E-32**	39,026	
					Replication	1.7	0.32 (0.24)	0.171	10,157	
					Combined	3.4	0.85 (0.07)	**1.17E-31**	49,183	0.050
rs148126992	12q14.3	*HELB*	p.Glu522Asp	C/G	Discovery	2.5	1.03 (0.09)	**2.96E-30**	38,707	
					Replication	0.1	2.16 (1.75)	0.216	10,157	
					Combined	2.5	1.04 (0.09)	**1.69E-30**	48,864	0.116
rs140267842	20q13.33	*SLCO4A1*	p.Val263Ile	A/G	Discovery	0.8	0.80 (0.16)	5.58E-07	39,026	
					Replication	1.2	0.73 (0.28)	8.60E-03	10,157	
					Combined	0.9	0.79 (0.14)	**1.60E-08**	49,183	0.241

Notes: Amino acid change is from the amino acid coded by the common allele to the amino acid coded by the minor allele. Significant p-values are in bold.

HELB is a DNA helicase that unwinds DNA during replication, transcription, repair and recombination. SLCO4A1 (solute carrier organic anion transporter family, member 4A1) transports organic anions such as thyroid hormones and estrone-3-sulfate. Both exome array signals in *HELB* and *SLCO4A1* were located in ANM loci newly identified by our parallel HapMap2 GWAS meta-analysis. At *HELB* the association of the common index SNP, rs12371165, was fully explained by associations at the two rare exome chip SNPs, which are in high LD with each other (r^2=0.73, D'=1) (Figure 2). In contrast, the three independent signal SNPs identified through GCTA were not explained by the rare variant(s) (Supplementary Table 8). It thus appears there are at least two non-redundant signals at this locus and future fine-mapping experiments will be required to fully elucidate the number of independent causal variants. Functional studies have shown that substitution of aspartate by a non-polar residue at amino acid 506 of *HELB* affects binding of HELB to Replication Protein A (RPA) [8]. At *SLC04A1*, all three variants (the common index SNP, second signal from GCTA and the exome chip variant) appeared to reflect non-redundant signals, such that the association of each with ANM was unaffected by the presence of either of the others (Supplementary Table 8).

Figure 2. Multiple signals at HELB and relationship to DNA helicase B protein sequence.

Positions are given in Build 37 coordinates of the reference genome. The top signal from the exome chip analysis maps to an acidic motif of DNA helicase B and results in the replacement of an acidic aspartate residue by a nonpolar glycine residue. Concurrent alteration of three acidic amino acids, (including the aspartate residue identified by the exome chip analysis) to nonpolar residues has been shown to reduce RPA binding[8].



ANM SNPs strongly enriched in DNA damage-response pathways

Pathway analyses using MAGENTA and GRAIL indicated substantial enrichment of GWAS SNP associations in DNA damage response (DDR) pathways (Supplementary Tables 9 and 10). Seven of the 10 ANM pathways identified by MAGENTA at study-wise significance were involved in DDR, with the highest enrichment in the PANTHER defined 'DNA Repair Pathway' ($P=1x10^{-6}$). After annotating likely causal genes at each locus, we found that 29 of the 44 GWAS highlighted regions contained one or more DDR genes within 500kb (Table 1). At 18 of these 29 regions, the DDR candidate was either the nearest gene or the signal was associated with expression of a DDR gene at the locus.

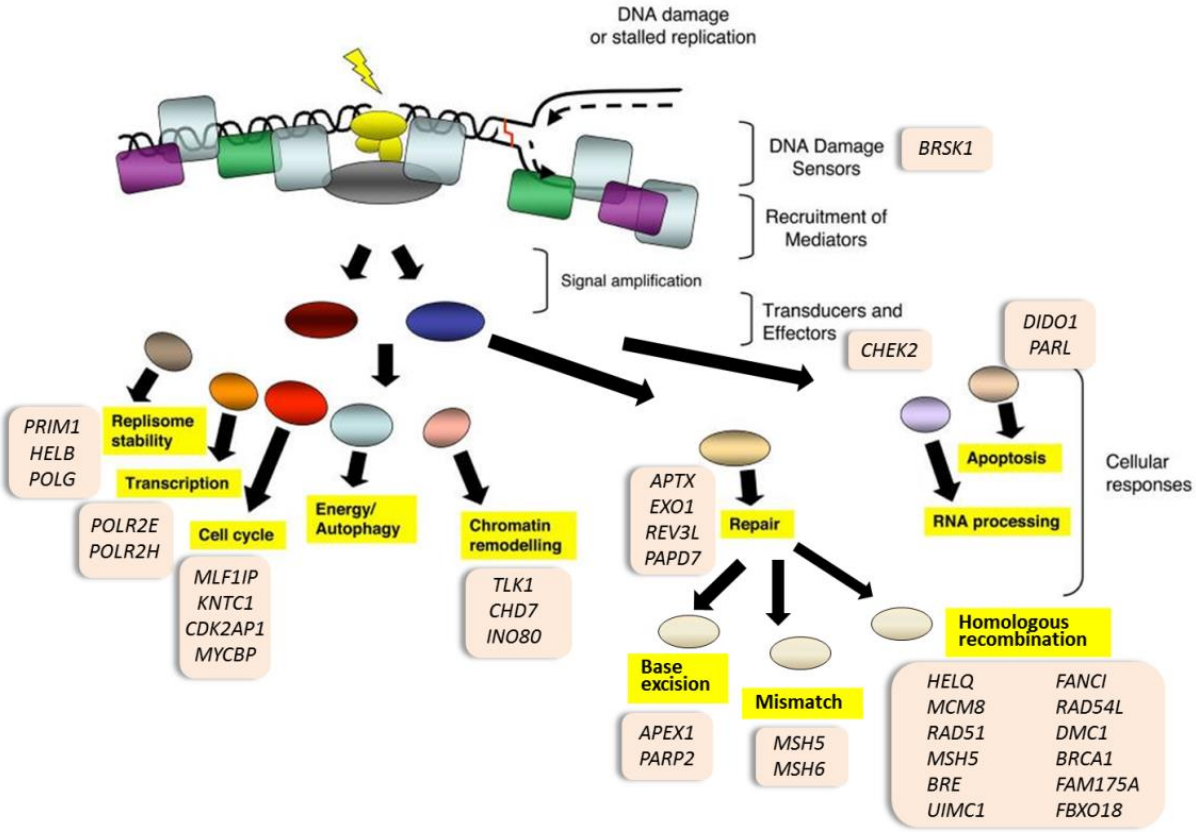
The top SNP at GWAS Signal #37 (Table 1) was highly correlated ($r^2>0.95$) with four common non-synonymous variants in *BRCA1* (rs1799966, rs16942, rs16941, rs799917), none of which is listed in HGMD (www.hgmd.cf.ac.uk/) as a known breast cancer susceptibility variant and all of which are listed as "not clinically important" on the Breast Information Core http://research.nhgri.nih.gov/bic/. In our exome array data, no low frequency coding variants in *BRCA1* were associated with ANM ($P>0.05$). Signal #37 was an eQTL for *BRCA1* in multiple tissues, including: blood, skin, adipose and brain (Supplementary Table 11). There were 15 ANM signal genes that STRING analysis identified as having at least one direct link to *BRCA1* (Supplementary Table 12, Supplementary Figure 2). Of these, there is experimental evidence that 7 code for direct binding partners of *BRCA1*: *BRE* (Signal #5), *MSH6* (Signal #6), *POLR2H* (Signal #8), *FAM175A* (Signal #9), *UIMC1* (Signal #13), *RAD51* (Signal #30), and *CHEK2* (Signal #43).

While many of the DDR genes highlighted are involved in homologous recombination for repair of double strand breaks, such as the *BRCA1* pathway, other mechanisms of repair are also represented, eg. mismatch repair (*MSH5*, *MSH6*) and base excision repair (*APEX1*, *PARP2*) (Figure 3). Two genes act as DNA damage checkpoints (*CHEK2* and *BRSK1*), others are involved in the cellular response to damage, such as cell cycle arrest, DNA replication, transcription control and apoptosis (Figure 3). *CHEK2* is a well-known breast cancer associated gene [9], but the ANM-associated signal was not in LD with the 1100delC variant associated with breast cancer ($r^2<0.01$).

ANM SNPs enriched in known POI genes

In addition to the DDR pathways, MAGENTA analyses also identified a four-fold enrichment of ANM GWAS SNP associations located in/near a set of 31 genes reportedly associated with monogenic primary ovarian insufficiency (Supplementary Tables 13 &14). Four of our genome-wide significant hits were located in or near reported POI genes. Autosomal recessive mutations in *MCM8* cause primary amenorrhea, hypothyroidism, and hypergonadotropic hypogonadism [10]. Recessive mutations in *EIF2B4* (signal #5) cause ovarioleukodystrophy with vanishing white matter syndrome [11]. *POLG* (signal #31) mutations have been linked to POI in isolation or associated with other neurologic conditions [12]. Mutations in *MSH5* (Signals #15a and #15b) have been associated with various human diseases including POI [13]. In addition, *TDRD3* (Signal #28) is a primary binding partner of *FMR1* in which triplet repeat premutations are a risk factor for POI [14]. We saw no significant enrichment of ANM signals in our wider panel of ovarian function genes (Supplementary Tables 13 and 15).

Figure 3. Classification of genes identified as being involved in the DNA damage response, at genetic loci associated with ANM.



Adapted from: Jackson & Bartek, Nature, 2009

Genetic correlation of ANM with other traits/diseases

We searched the GRASP database [15] and NHGRI catalogue (http://www.genome.gov/gwastudies/) for pleiotropy between ANM signals and proxies (r2>0.5) with other GWAS traits (Supplementary Table 16). The top overlapping signals were for liver enzymes, lipids, urate, height and fasting glucose ($p=<10^{-10}$ for association of ANM SNP/proxy and second trait). We found no overlap with any autoimmune traits and only a very weak link with any cancer (upper airway tract cancer, $p=1x10^{-8}$). To test the relationship between ANM and other health outcomes more broadly, we performed cross-trait LD score regression to estimate genetic correlation with 53 published GWAS meta-analyses (Supplementary Table 17). Adult obesity ranked highest in this analysis with a negative trait correlation (r_g=-0.15, P=0.0004) with supporting evidence from other growth/anthropometric traits including age at menarche (r_g=0.14, P=0.003), BMI (r_g=-0.13, P=0.003), BMI in women but not men (P=0.002 vs 0.17), waist circumference in women but not men (P=0.009 vs 0.29) and WHR in men but not women (P=0.03 vs 0.27). Other nominally significant associations include HDL (r_g=0.14, P=0.02) and current/former smoking status (r_g=0.20, P=0.04) both of which are supported by epidemiological observations [16].

To elucidate the causal directions between these traits, we performed bi-directional Mendelian randomisation (MR) analyses on ANM with both age at menarche and BMI. We were unable to resolve the causal direction with BMI (BMI to ANM: P_{score}= 0.668 (Supplementary Table 18); ANM to BMI: $P_{Binomial}$=0.683, (Supplementary Table 19). However the 123 reported menarche SNPs collectively predicted ANM in the expected direction (P_{score}=0.0005, Supplementary Table 20), but the ANM SNP score was not associated with age at menarche (P_{score} = 0.571, Supplementary Table 21). We further explored the nature of this shared genetic architecture by testing for enrichment of all ANM-associated SNPs in/near genes implicated in monogenic or polygenic puberty timing [17]. Significant enrichment was found with the monogenic set (P=0.01), underscored by ANM-associated SNPs in/near five genes reportedly causal for hypogonadotrophic hypogonadism (*KISS1R, TAC3, CHD7*, *SOX10* and *FGFR1*) (Supplementary Table 22).

ANM variants demonstrate causal link with breast cancer

Given the overwhelming enrichment of DDR genes and known epidemiological associations between ANM and breast cancer risk [18], we tested the causal relationships between these traits using a Mendelian Randomization approach [19].

Across the 56 ANM SNPs (54 HapMap 2 + 2 exome) there was a positive correlation between the effect sizes on ANM and the effect sizes for risk (logORs) of breast cancer (in 46,347 breast cancer cases and 41,736 controls from Breast Cancer Association Consortium (BCAC); r=0.67, P=2.25x10-8). A polygenic risk score comprising numbers of ANM-increasing alleles at the 56 SNPs, weighted by the effect size on ANM, was positively associated with breast cancer risk; each one-year older genetically predicted ANM was associated with a OR=1.064 higher breast cancer risk (1.050-1.081), P=2.78x10-14 (Supplementary Figure 3). This effect size is larger than that reported by the largest pooled analysis of observational epidemiological studies (OR=1.030 (1.026-1.034)) [18]. All of the women in the GWAS from the BCAC study were also included in the Mendelian randomization (MR) study (N=14884, ~14% of total MR study). To confirm that this overlap did not bias our results we conducted two analyses. Firstly, a sensitivity analysis tested the effect on breast cancer of 18 previously identified ANM SNPs, which were identified from a meta-analysis that did not include BCAC cases, and a similar effect estimate was observed (OR 1.062 [1.033-1.101, P=1.58x10-7]) Secondly, the reverse analysis tested 63 SNPs with independent robust associations with breast cancer [20], and found no association between these breast cancer signals and ANM (Pscore >0.05), which reduces the likelihood of case-ascertainment bias in our discovery meta-analysis (Supplementary Table 23).

Stratified analyses revealed significantly larger effect estimates for the ANM risk score in ER positive vs ER negative breast cancer cases (OR=1.07 (1.05-1.10) P=1.73x10-12 vs OR=1.03 (1.00-1.07) P=0.043; P=0.0086 for the case-only analysis) and women aged >=55 vs <=45 years (OR=1.06 (1.04-1.10) P=2.23x10-7 vs OR=1.00 (0.97-1.05) P=0.95, case-only P=2.30x10-5). Consideration of DDR vs non-DDR linked SNPs in the polygenic risk score also produced discordant effect estimates (OR 1.05 [1.03-1.08], p=1.06x10-7 vs OR 1.12 [1.06-1.21], P=7.84x10-10 respectively, Phet=0.01), a difference which

was further reinforced in the age stratified analyses (Supplementary Figure 3 and Table 24).

Furthermore, lack of association between ANM risk scores with risk of prostate cancer in men (in 25,074 cases and 24,272 controls) (P=0.36, Supplementary Table 25) provides no evidence to support an effect of ANM-related DDR mechanisms on other cancer risks. We therefore surmise that ANM genetic variants influence breast cancer risk primarily through variation in menopause timing.

Discussion

Our study represents a largely expanded genetic discovery effort for ANM, both in terms of increased sample size and breadth of variation tested. By more than doubling the GWAS sample size we have increased the number of loci robustly associated with the trait three-fold. In addition, we assessed the role of low-frequency protein coding variation using exome genotyping arrays. This approach identified the first such variants of large effect for ANM, implicating both *HELB* and *SLC04A1* in the aetiology of reproductive ageing. Both of these regions contain common variants we identified in parallel, producing “synthetic associations” at the *HELB* locus [21].

Our analyses suggest a far more substantial role for DNA damage response processes in ovarian ageing than originally estimated. Both manual assessment and formal computational approaches identified an overwhelming excess of DDR genes mapping to the 44 GWAS loci, possibly explaining up to ~2/3rds of the associations. Despite the limitations of our GWAS approach to map definitively SNPs to genes, 19/44 loci contained signal SNPs where plausible DDR candidates were either the closest gene or linked via altered expression levels to the associated variant. This level of enrichment is comparable to that observed in GWAS meta-analyses of several cancers [22,23].

A notable inclusion in our list of DDR annotated genes was *BRCA1*, which was the nearest gene, linked as an eQTL and contained multiple non-synonymous SNPs in high LD with the lead index SNP. Although rare loss of function alleles are well studied in the context of cancer pre-disposition, coding variants in *BRCA1* are generally regarded as neutral and have not been previously

mapped to any complex trait or disease, including breast cancer. Titus *et al* have shown that *BRCA1* expression decreases in human ovaries with age and that reduced *BRCA1* expression in mouse models leads to reduced ovarian reserve [24]. This is consistent with our data, where the ANM-lowering allele reduces expression in blood. BRCA1 directly inhibits a functional interaction with oestrogen receptor α and thus *BRCA1* variants could also affect ANM through altered oestrogen signalling [25]. Of the 34 DDR genes highlighted in Table 1, 15 have experimental links to *BRCA1*, three of which form part of the BRCA1-A complex; *BRE* (BRCC45), *FAM175A* (Abraxas) and *UIMC1* (RAP80). While dispensable for BRCA1's major tumour suppressive role in promoting DNA double-strand break repair by homologous recombination (HR), the BRCA1-A complex components RAP80 and Abraxas are actually involved in counteracting this activity, restricting BRCA1-dependent HR to appropriate levels [26]. Similarly, the DNA helicase Fbh1 (*FBX018*; Signal #20) negatively regulates HR [27,28]. While HR is essential for cell viability, such anti-recombinase activities are also important for maintaining genome stability, and failure of this regulation is associated with inappropriate recombination events, and the accumulation of toxic recombination intermediates, DNA repair activities associated with driving translocations, loss-of-heterozygosity, and chromosomal abnormalities [29].

Double strand break repair is an important response to metabolic and environmental damage to DNA, but is also a key process in meiosis for resolving recombination events. Aberrant meiotic recombination is known to cause meiotic arrest and affect the viability of oocytes. Menopause occurs when the number of oocytes in the ovary falls below a threshold number (approx. 1000) and thus processes that affect the size of the oocyte pool will affect timing of menopause. Recent studies have shown that recessive mutations in both *MCM8* and *MCM9* results in genomic instability, caused by a deficiency in double strand break repair, which has a devastating effect on the oocyte pool, causing POI [10,30]. *MCM8* is one of the genes highlighted in our study (signal #41) and a further 12 are also involved in homologous recombination repair, including two which are specific for meiotic repair (*MSH5* and *DMC1* (DNA meiotic recombinase 1)). Thus double strand break repair, during

recombination, at meiosis, appears to be a major mechanism by which oocyte numbers are regulated, thus determining depletion of the oocyte pool and ANM.

In this study, however, the repair mechanisms highlighted are not confined to homologous recombination repair; mismatch repair and base excision repair are also implicated, as well as mitotic repair and repair checkpoints. Thus it appears that the mechanisms are not confined to repair of meiotic cross-overs, but more general mechanisms are also involved. Seven million oogonia are produced during fetal development by mitosis. Inefficient repair of DNA damage during these mitotic events could result in apoptosis and thus a reduction in the initial oocyte pool. Loss of oocytes throughout female life is predominantly by atresia rather than ovulation. It is likely that oocytes are particularly sensitive to DNA damage due to the prolonged state of cell cycle arrest, lasting up to 50-60 years. Thus aberrant repair throughout life could affect the rate of atresia and thus ANM.

Several of the genes highlighted in our study are robust cancer predisposition genes, eg. *BRCA1*, *CHEK2* and *MSH6*. Additionally *BCAR4* and *STARD2* have also been linked with breast cancer predisposition. However common susceptibility variants have not been mapped to any of these genes through GWAS approaches for any cancer [www.genome.gov/gwastudies/]. Patients with known pathogenic *BRCA1* breast cancer predisposition mutations, have been reported to have lower ANM [31], although other studies have failed to replicate these findings [32].

We found that carrying higher numbers of ANM-increasing variants was associated with increased breast cancer risk. This was consistent with (indeed slightly larger than) the observed epidemiological association. Our Mendelian randomization approach indicates a causal relationship between ANM and breast cancer risk, with prolonged oestrogen and/or progesterone exposure likely to be the mechanism [33]. Consistent with this, the effect size was greater for ER-positive than ER-negative breast cancer.

At first sight, this observation might appear paradoxical given the enrichment of DDR genes associated with menopause. However, we noted that the association between ANM variants and breast cancer risk was weaker for those in/near DDR genes than those in the non-DDR set. This raises the possibility

that the DDR variants that reduce menopausal age do modestly increase breast cancer risk, but this is counterbalanced by the larger effect due to altered hormonal exposure. Alternatively, it is possible that variants in the non-DDR set may have a residual effect on breast cancer risk through hormonal or other mechanisms, or that both mechanisms could play a role (supplementary Figure 4). *BRCA1* mutations are known to be risk factors for prostate cancer [34] and yet we found no association with prostate cancer predisposition for the ANM variants, supporting the hypothesis that the breast cancer association is mediated via menopause and not a direct effect of the DDR variants. That the effect of the ANM polygenic risk score on breast cancer risk was larger than that predicted from observation studies might indicate measurement error in the reporting of age at menopause or residual negative confounding in epidemiological studies; in either case, the Mendelian Randomisation analysis performed here using the polygenic risk score as an instrumental variable can give a more accurate estimate of the effect of age at menopause on breast cancer risk. Such measurement error would also be present in studies in the ANM GWAS from which the polygenic risk score weights were derived, hence the 'true' effect of later menopause on breast cancer risk may actually be larger even than the ~6% increase in risk/year predicted here.

Our findings provide novel evidence for a neural influence on the timing of ovarian follicular ageing. Until now, it has been considered that hypothalamic/pituitary activity in relation to the menopause is simply secondary to the loss of feedback inhibition by ovarian hormones [35]. We identified five ANM loci containing genes reported causal for hypogonadotrophic hypogonadism. Of these, monogenic disruption of three (*CHD7*, *FGFR1* and *SOX10*) are causes of Kallman syndrome, characterized by anosmic hypogonadotrophic hypogonadism due to failure of embryonic migration of GNRH secreting neurons from the olfactory bulb to the hypothalamus [36]. In addition, *KISS1R* (*GPR54*) encodes the receptor for kisspeptin, a key hypothalamic activator of the reproductive hormone axis, and *TAC3* encodes neurokinin B, which is highly expressed in hypothalamic neurons that also express kisspeptin and promotes the pulse frequency of luteinising hormone (LH) secretion from the pituitary. A possible central influence on ovarian ageing is also supported by the ANM locus in/near *FSHB* (which is reportedly also

associated with circulating FSH levels). Alternatively, recent studies have identified expression of TAC3, KISS1R and kisspeptin in ovarian granulosa cells [37], suggesting peripheral actions of these neuropeptides and their receptors [38]. Indeed, *GPR54*-haploinsufficiency in mice leads to progressive oocyte and follicle loss without affecting gonadotropin secretion [38]. Regardless of their site of action, our findings indicate several mechanisms that could link the regulation of puberty to ANM, and therefore impact both the start and end of the female reproductive lifespan.

In summary, our findings suggest a surprisingly narrow range of biological pathways governing ANM, highlighting a substantial role for DNA damage response in the aetiology of ovarian ageing. We demonstrate the utility of genetics to inform epidemiological observations, revealing shared biological pathways linking puberty timing, breast cancer and reproductive ageing.

URLS

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http://www.hgmd.cf.ac.uk/
http://research.nhgri.nih.gov/bic/
http://www.genome.gov/gwastudies/)
http://www.chargeconsortium.com/main/exomechip
http://string-db.org/
http://www.broadinstitute.org/mpg/snap/
https://github.com/bulik/ldsc
http://www.1000genomes.org/
https://github.com/bulik/ldsc

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See supplementary information

Author contributions

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Statistical analysis

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Sample collection, genotyping and phenotyping

G.A, I.L.A, H.A, A.C.A, V.A, A.M.A, C.Ba, M.W.B, A.B-F, J.B, L.B, S.J.B, C.Bl, E.B, N.V.B, S.E.B, M.K.B, A.B-D, T.S.B, H.Bra, H.Bre, T.B, B.B, A.Ca, H.C, S.J.C, J.R.C, Y.C, G.C, F.J.C, A.D.C, A.Co, K.C, H.D, I.DV, E.W.D, J.D, P.D, T.D, I.dSS, A.M.D, J.D.E, P.A.F, J.D.F, J.F, D.F, I.G, M.E.G, M.G, G.G.Giles, G.G.G, M.S.G, A.G, M.O.G, M.L.G, D.F.G, P.G, X.G, C.A.H, P.H, U.H, B.E.H, L.J.H, A.H, G.H, M.J.H, J.L.H, F.B.H, J.H, K.H, D.J.H, A.J, M.K, D.K, J.A.K, I.K, C.K, V.Ko, J.K, V.Kr, D.L, C.L, J.Li, X.L, S.L, Y.L, J.Lua, J.Lub, R.M, A.Ma, J.Manz, S.M, J.M, N.G. M, C.M, A.Mei, K.M, E.M, L.M, R.L.M, M.Mü, M.N, B.M.N, H.N, P.N, A.B.N, B.G.N, J.E.O, S.P, P.P, U.P, A.Pe, J.P, P.D.P.P, N.N.P, A.Pi, G.P, O.P, D.P, B.M.P, K.P, P.R, L.J. R, F.R, I.R, A.Ru, D.R, C.F.S, S.S, E.J.S, D.Sc, M.K.S, F.S, R.K.S, M.J.S, R.A.S, C.MS, J.S, R.S, M.C.S, D.St, K.Str, A.S, K.D.T, U.T, A.E.T, I.T, T.Tr, L.T, S.T.T, D.Vo, Q.W, M.W, G.W, J.F.W, R.W, B.B.H.R.W, A.F.W, D.Y, T.Z, W.Z, M.Z

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Data will be made available on the *ReproGen* web site (www.reprogen.org)

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Online Methods

Menopause data collection

ANM was self-reported and defined as the age at last naturally occurring menstrual period followed by at least 12 consecutive months of amenorrhea. Recall bias/error for ANM may have reduced our power to detect associations, but would be unlikely to introduce systematic error. We assessed this issue in our previous meta-analysis and found no significant differences in effect estimates when considering retrospective versus prospective studies [4]. We included women with ANM 40–60 years in our analyses, excluding those with menopause induced by hysterectomy, bilateral ovariectomy, radiation or chemotherapy, and those using hormone replacement therapy (HRT) before menopause (Supplementary Table 1). Within each of the included studies, each participant provided written informed consent and the study protocol was approved by the Institutional Review Board at the parent institution.

GWAS

A total of 33 studies contributed genome-wide association data using self-reported ANM (Supplementary Table 1). One of the 33 studies was from the Breast Cancer Association Consortium (BCAC), comprising 17 separate studies with menopause data, genotyped using an Illumina iSelect array (iCOGs) [20]. This resulted in a maximum total sample of 69,360 individuals of European descent. Studies were asked to use the full imputed set of HapMap Phase 2 autosomal SNPs, and to run an additive model including top principal components and study specific covariates.

In some cases, studies submitted data using 1000 Genomes based imputation; in these cases SNPs not included in the HapMap 2 set were removed. Once data were submitted, each study was quality controlled centrally according to standard QC protocols independently by two analysts. SNPs were filtered out if the minor allele frequency (MAF) was less than 1%, or if the imputation quality metrics were low (imputation quality<0.4). Studies and SNPs passing QC were combined using an inverse-variance weighted meta-analysis, implemented using METAL [39]. Again, this meta-analysis was run by two analysts independently, who then separately used PLINK clumping commands [40] to

identify the most significant SNPs in associated regions (termed “Index SNPs”), using only those SNPs which had data from more than 50% of the studies. SNPs were considered genome-wide significant if $p<5\times10^{-8}$ (p of 0.05 Bonferroni corrected for a million tests). Comparisons were made to ensure concordance of the identified signals between the two independent analysts.

Exome chip

Exome genotyping data were analysed for 22 studies of European ancestry, with questionnaire data on ANM (Supplementary Table 6). Genotype calling was performed using the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) joint calling protocol, including X chromosome variants. Each contributing study carried out study-level analysis in the R-packages skatMeta or seqMeta using the skatCohort command with the top genetic principal components included in the model and alleles coded according to a common reference file (SNPInfo_HumanExome-12v1_rev5.tsv.txt from http://www.chargeconsortium.com/main/exomechip) [41]. Following data submission, two data analysts carried out checks to ensure consistency of allele coding. We carried out a single variant meta-analysis in METAL [39], giving a total sample size of 39,026, with associations considered significant if $p<5\times10^{-8}$. Variants were put forward for replication in the deCODE study (n=10,157) if they were present in more than half of studies in the discovery stage and had $p<5\times10^{-5}$ if MAF was less than 1% or $p<5\times10^{-4}$ if MAF was 1–5%.

Selection of independent signals / conditional analysis

Independent signals (termed “Signal SNPs”) for ANM were identified using approximate conditional analysis implemented in the GCTA software package [42]. Linkage disequilibrium (LD) between variants was estimated using three independently genotyped studies as reference panels - the Rotterdam Study I (N=5,974) and two EPIC-InterAct datasets (N=7,397 and N=9,294); these comprised males and females of European ancestry with GWAS data imputed using CEU haplotypes from HapMap 2. We assumed zero correlation between SNPs more than 10 Mb apart or on different chromosomes. We considered independent signals to be those observed by at least two of the three LD reference panels and located in a 10 Mb region that contained a genome-wide significant SNP based on univariate test statistics.

We assessed the independence between exome array and HapMap 2 signals by performing formal conditional analyses in the Women's Genome Health Study (WGHS, N=11,664). Regression was performed including all significant index SNPs in additive models, including the same study covariates as used in the primary analysis. LD computation in Haploview [43]used experimental genotypes where possible (the rare exome chip variants and the common variants rs3741604 and rs2236553), but HapMap 2 imputed genotypes for the other common variants (MaCH v. 1.0.16, all Rsq >0.99).

Gene identification

At each locus identified by the GWAS meta-analysis, we annotated the likely causative gene(s) (Supplementary Table 3) using the following criteria: identified in at least one of the gene prioritisation/pathway programs (GRAIL or STRING), the top SNP or a proxy (r^2>0.8) was an eQTL in one of 108 tissues, the top SNP or a proxy (r^2>0.8) was a coding variant (Supplementary tables 9-12, 26, 27, Supplementary Figure 5). In case of overlap between the results of the GWAS and exome analyses, the gene indicated by the exome array analysis was chosen. Further manual annotation was used to select additional likely candidates based on known biology (e.g monogenic primary ovarian insufficiency) or biology highlighted by hypothesis-free pathway testing (Supplementary Table 15). If no candidate was identified by these methods the nearest gene was chosen.

GRAIL is a literature based text mining program used to suggest the mostly likely casual gene at each locus [44], controlling for gene size and without any seed regions. A GRAIL p-value < 0.05 was taken to indicate a suggested causal gene (Supplementary Table 9). All genes located within 500kb of the top SNP at each locus were assessed using the STRING program (http://string-db.org/), which was used to highlight any connectivity between genes in different regions (Supplementary Table 12).

Expression quantitative trait loci (eQTL)

Each independent SNP signal was assessed in over 100 separate eQTL datasets (Supplementary methods and Table 11 for details 45). If an independent signal SNP was in high LD (r2>0.8; using SNAP

http://www.broadinstitute.org/mpg/snap/) with the most significant signal for an eQTL, then the eQTL gene was highlighted as a potential causal candidate. The collected eQTL results met criteria for statistical thresholds for association with gene transcript levels as described in the original papers.

Pathway identification

We tested for signal enrichment across 2,580 pre-defined biological pathways in GO, KEGG, Ingenuity, Panther, Reactome and Biocarta using MAGENTA [46] using the full HapMap Phase 2 imputed meta-analysis (Supplementary Table 10). Analysis was performed using the same default settings as described in our previous paper [4], with study-wise significance declared at an FDR<0.05. In addition to these pre-defined pathways, we also tested four custom pathways comprised of genes involved in POI (N=31), ovarian function (N=130), monogenic disorders of puberty (N=21) and age at menarche (N=154) (Supplementary Tables 13-15, 22).

Estimating variance explained by SNP sets

An estimate of the total variance explained by highlighted ANM SNPs was calculated using REML (restricted maximum likelihood) implemented in GCTA [42]. Using individual level data from the EPIC-InterAct cohort (N=1,761), we calculated the attributable variance for the genome-wide significant SNPs and at varying significance thresholds (5×10^{-7}, 5×10^{-6}, 5×10^{-5}, 5×10^{-4}, 0.005, 0.05, and all SNPs passing QC) obtained from a repeated meta-analysis excluding EPIC-InterAct.

We used stratified LD score regression to quantify evidence of functional enrichment specific to groups of cell types [6]. We used the same baseline model as in Finucane et al. [6] which comprises 53 overlapping categories including basic annotations such as coding, UTR, promoter, and intron, as well as several histone marks, DNase I Hypersensitivity Site (DHS) regions, chromHMM predictions [47], regions that are conserved in mammals [48], super enhancers [49], and FANTOM5 enhancers [50]. We evaluated enrichments for each of these non-cell-type specific categories. We then took 230 cell-type-specific annotations in four histone marks-H3K4me1, H3K4me3, H3K9ac [51] and H3K27ac [52] (Supplementary Table 5), and grouped them into 10 cell-type groups,

(adrenal/pancreas; central nervous system; cardiovascular; connective/bone; gastrointestinal; immune/hematopoietic; kidney; liver; skeletal muscle; other) [6]. We added each cell-type group to the baseline model one at a time and measured the p-value of the resulting LD Score regression coefficient of the cell-type group using the –h2 flag in ldsc (https://github.com/bulik/ldsc) with LD Scores from 1000G Genomes Europeans [http://www.1000genomes.org/]. We ranked the cell-type groups by whether the per-SNP heritability in the 'functional' annotation was larger than the per-SNP heritability outside this annotation, controlling for the other annotations in the baseline model.

Breast and prostate cancer Mendelian Randomisation (MR)

To assess the association of the ANM SNPs with breast cancer risk, we used breast cancer cases (n=46,347) and controls (n=41,736) of European ancestry from 41 studies in the BCAC, who had been genotyped using a custom Illumina Infinium array (iCOGS). Following standard quality control exclusions (as described in [20]) genotypes were available for 199,961 SNPs. Further genotypes were imputed in a two-stage procedure using SHAPEIT and IMPUTEv2 [53] with the 1000 Genomes Project March 2012 release as the reference dataset [54], giving ~11.6 million SNPs with imputation r^2>0.3 and MAF>0.005. The 4,747 breast cancer cases and 7,285 controls in the BCAC dataset for whom ANM information was available had also been included in the ANM GWAS analysis.

The genotypes or imputed genotype dosages for the 56 significant SNPs in Tables 1 and 2 were used to construct a polygenic risk score for each breast cancer case and control, such that for the i^{th} woman

$$PRS_i = \sum_{j=1}^{56} \beta_j G_{ij}$$

where β_j is the ANM regression coefficient for the effect allele of the j^{th} SNP (conditional βs were used for the correlated SNPs) and G_{ij} is the number of copies of the effect allele at the j^{th} SNP carried by the i^{th} woman (G_{ij} is between 0 and 2).

The association between the polygenic risk score and breast cancer was tested using unconditional logistic regression, adjusting for study and for seven principal components (as estimated based on a subset of 37,000 uncorrelated

markers including ~1000 selected as ancestry informative markers). The log(OR) was scaled according to the effect size of a one-unit increase in polygenic risk score on ANM in control subjects, so as to obtain an estimated logOR for a one-year increase in genetically predicted ANM. Hence the polygenic risk score can be thought of as an instrumental variable in a Mendelian Randomisation of ANM against breast cancer.

Additional analyses were conducted specifically for estrogen receptor (ER) positive (N=27,026) or ER negative (N=7,401) cases, and for participants with age at diagnosis (for cases) or interview (for controls) ≤45 years (8,547 cases and 8,029 controls) or ≥55 years (24,841 cases and 20,410 controls)(as a surrogate for pre- or post-menopausal age at diagnosis, because ANM was not known for all participants), with heterogeneity evaluated in case-only analyses.

We also tested the association of ANM SNPs on prostate cancer risk, to determine whether any effect of genetic variants was specific to breast cancer. Prostate cancer data were available from a similar sample size to breast cancer and there is known overlap in genetic risk for breast and prostate cancer. Individual level data was not available for prostate cancer, we therefore assessed the impact of ANM using an approximated allele score comprised of the 54 HapMap2 GWAS SNPs on summary level results [55]. The score was assessed using summary statistics from a recent prostate cancer meta-analysis, comprising 25,074 cases and 24,272 controls from 32 studies in the PRACTICAL Consortium [56], genotyped using the iCOGs array, with quality control and imputation carried out in the same way as for the BCAC iCOGs study.

Genetic correlation with additional traits

Cross-trait LD score regression was used to estimate the genetic correlation between menopause timing and 54 individual traits from published studies including anthropometric and metabolic traits [57]. We estimated genetic correlations with the method described in [58] and the --rg flag in the ldsc software package (https://github.com/bulik/ldsc) with LD Scores from 1000 Genomes Europeans and default settings. Briefly, this method regresses the product of effect size estimates for trait 1 and trait 2 for each SNP against LD Score. The product of the slope and a constant estimates the genetic correlation, and the

intercept estimates the product of the number of overlapping samples and the correlation between phenotypes among the overlapping samples.

Bi-directional Mendelian randomisation analyses on ANM with age at menarche and BMI were carried out using similar methods as for prostate cancer, with a weighted allele score [55] generated from summary statistics. Information on the associations with age at menarche came from the most recent genome-wide association study for the trait (N=182,416 women from 57 studies) [17]. The BMI data were taken from the most recent analysis (N=249,796 from 64 studies) [59]. While it was possible to calculate a full allele score for the genome-wide significant BMI SNPs to ANM analysis, this was not possible for the ANM SNPs to BMI analysis; instead a binomial test of consistency of effect direction was used.

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There are no competing financial interests.

Supplementary Methods

Expression quantitative trait loci (eQTL) analysis

Blood cell related eQTL studies included fresh lymphocytes (17873875), fresh leukocytes (19966804), leukocyte samples in individuals with Celiac disease (19128478), whole blood samples (18344981, 21829388, 22692066, 23818875, 23359819, 23880221, 24013639, 23157493, 23715323, 24092820, 24314549, 24956270, 24592274, 24728292, 24740359), lymphoblastoid cell lines (LCL) derived from asthmatic children (17873877, 23345460), HapMap LCL from 3 populations (17873874), a separate study on HapMap CEU LCL (18193047), additional LCL population samples (19644074, 22286170, 22941192, 23755361, 23995691, 25010687), CD19+ B cells (22446964), primary PHA-stimulated T cells (19644074, 23755361), CD4+ T cells (20833654), peripheral blood monocytes (19222302,20502693,22446964) and CD14+ monocytes before and after stimulation with LPS or interferon-gamma (24604202), CD11+ dendritic cells before and after *Mycobacterium tuberculosis* infection (22233810) and a separate study of dendritic cells before or after stimulation with LPS, influenza or interferon-beta (24604203). Micro-RNA QTLs (21691150) and DNase-I QTLs (22307276) were also queried for LCL.

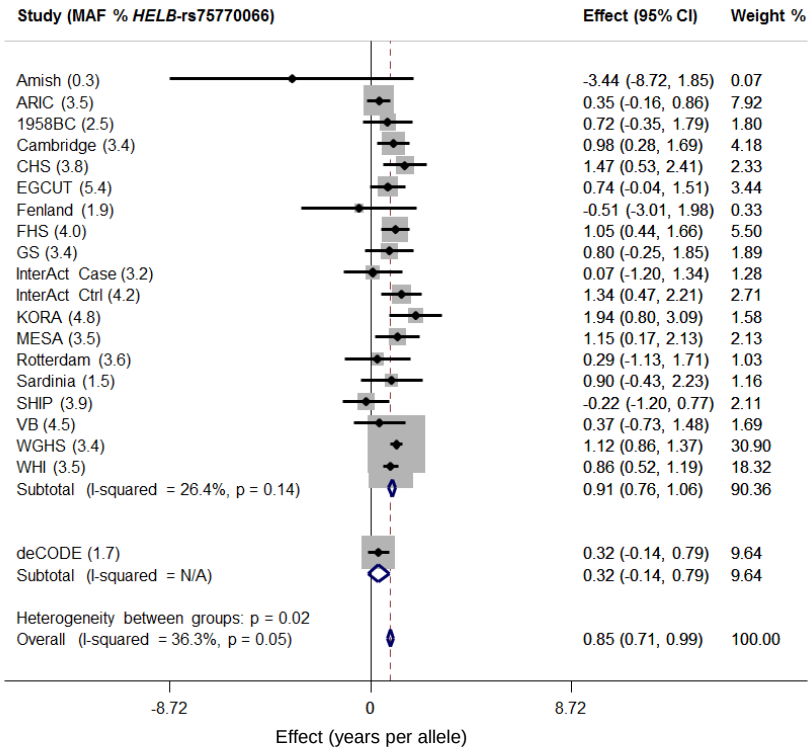
Non-blood cell tissue eQTLs searched included omental and subcutaneous adipose (18344981, 21602305, 22941192, 23715323), stomach (21602305), endometrial carcinomas (21226949), ER+ and ER- breast cancer tumor cells (23374354), liver (18462017,21602305,21637794, 22006096, 24665059), osteoblasts (19654370), intestine (23474282) and normal and cancerous colon (25079323), skeletal muscle (24306210), breast tissue (normal and cancer)(24388359, 22522925), lung (23209423, 23715323, 24307700), skin (21129726, 22941192, 23715323), primary fibroblasts (19644074, 23755361, 24555846), sputum (21949713), pancreatic islet cells (25201977) and heart tissue from left ventricles (23715323, 24846176) and left and right atria (24177373). Micro-RNA QTLs were also queried for gluteal and abdominal adipose (22102887) and liver (23758991). Further mRNA and micro-RNA QTLs were queried from ER+ invasive breast cancer samples, colon-, kidney renal clear-, lung- and prostate-adenocarcinoma samples (24907074).

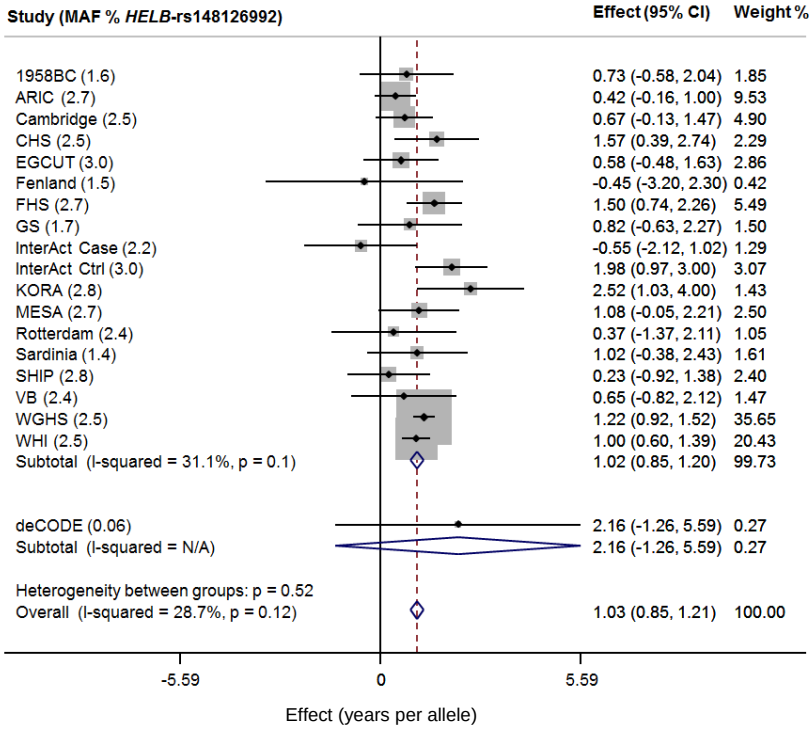
Brain eQTL studies included brain cortex (19222302, 19361613, 22685416), cerebellar cortex (25174004), cerebellum (20485568, 22685416, 22212596, 22832957, 23622250), frontal cortex (20485568, 22832957, 25174004), gliomas (24607568), hippocampus (22832957, 25174004), inferior olivary nucleus (from medulla) (25174004), intralobular white matter (25174004), occiptal cortex (25174004), parietal lobe (22212596), pons (20485568), pre-frontal cortex (22031444, 20351726, 22832957, 23622250), putamen (at the level of anterior commussure) (25174004), substantia nigra (25174004), temporal cortex (20485568, 22685416, 22832957, 25174004), thalamus (22832957) and visual cortex (23622250).

Additional eQTL data was integrated from online sources including ScanDB, the Broad Institute GTex browser, and the Pritchard Lab (eqtl.uchicago.edu). Cerebellum, parietal lobe and liver eQTL data was downloaded from ScanDB and cis-eQTLs were limited to those with P<1.0E-6 and trans-eQTLs with P<5.0E-8. The top 1000 eQTL results were downloaded from the GTex Browser at the Broad Institute for 9 tissues on 11/26/2013: thyroid, leg skin (sun exposed), tibial nerve, tibial artery, skeletal muscle, lung, heart (left ventricle), whole blood, and subcutaneous adipose (23715323). All GTex results had associations with P<8.4E-07.

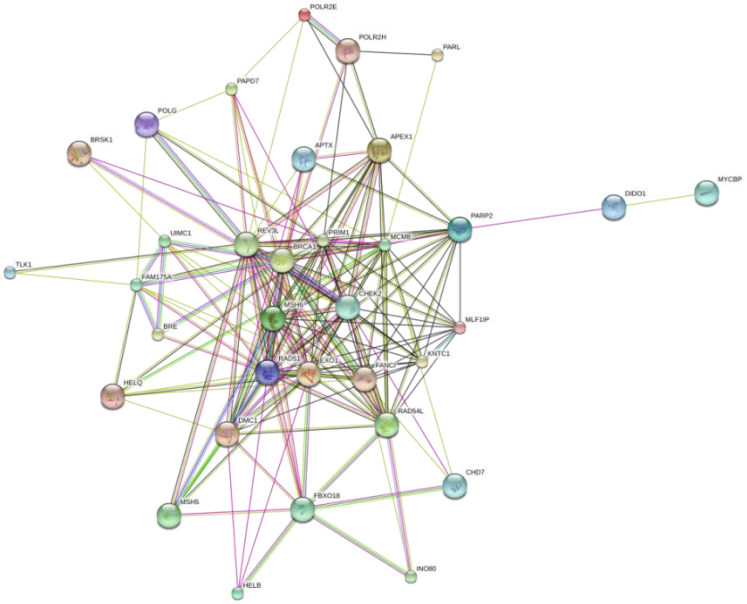
Supplementary Figures

Supplementary Figure 1. Study-specific test statistics and allele frequencies for the exome-chip variants in HELB.

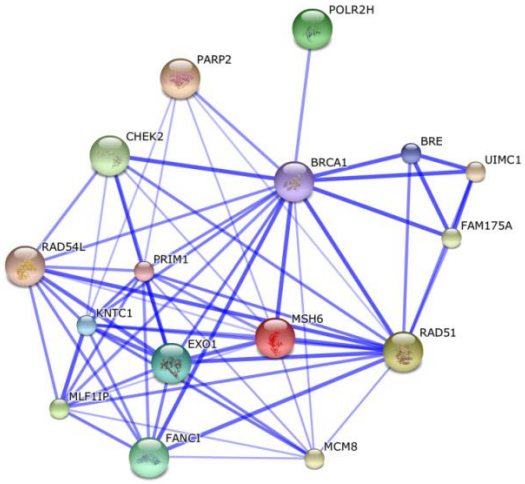




Supplementary Figure 2. STRING analysis of genes highlighted from GWAS. A. Connections for 34 genes highlighted as being involved in DDR at loci associated with age at menopause. B. Genes that are directly linked to BRCA1 from the list of highlighted genes in Table 1. Weight of connecting line indicates the strength of the evidence for the connection.

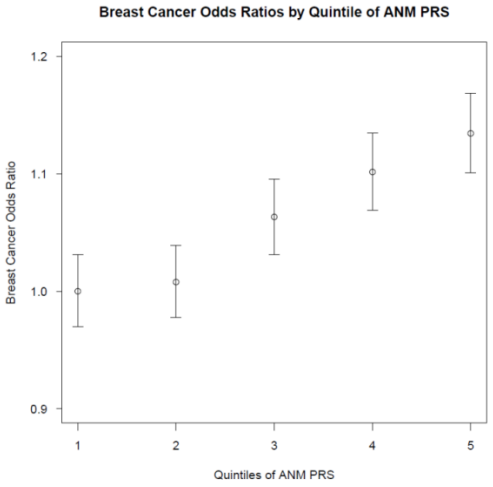


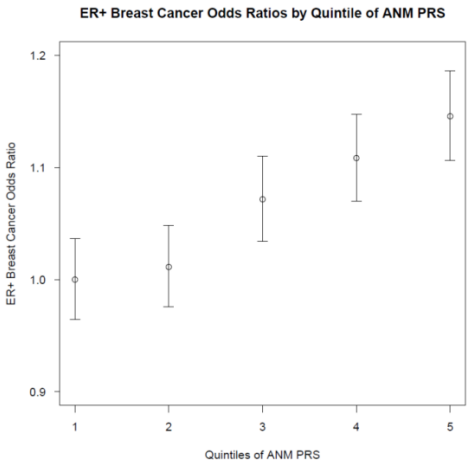
A

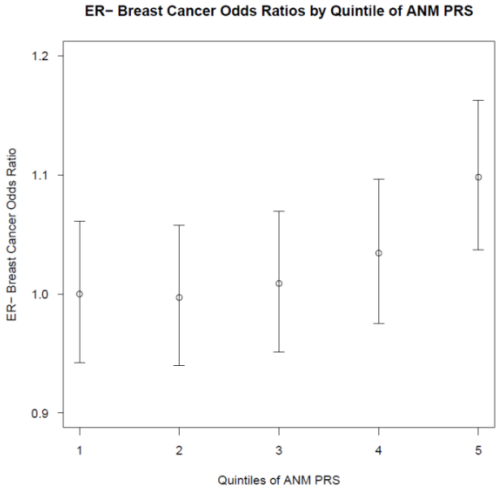


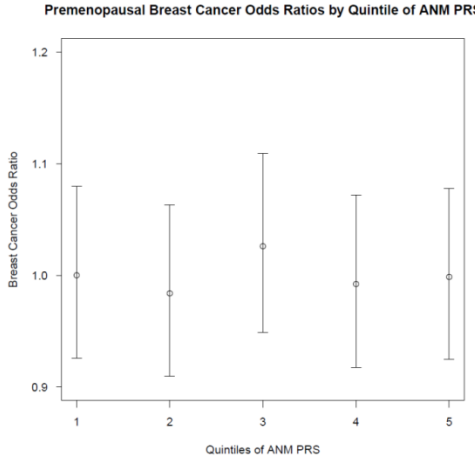
B

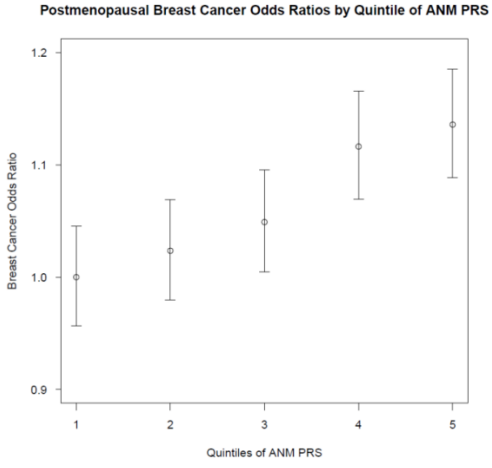
Supplementary Figure 3. Breast cancer ORs by quintile of ANM polygenic risk score (PRS) (quintiles defined in BC controls). The CIs are floating confidence intervals[60].



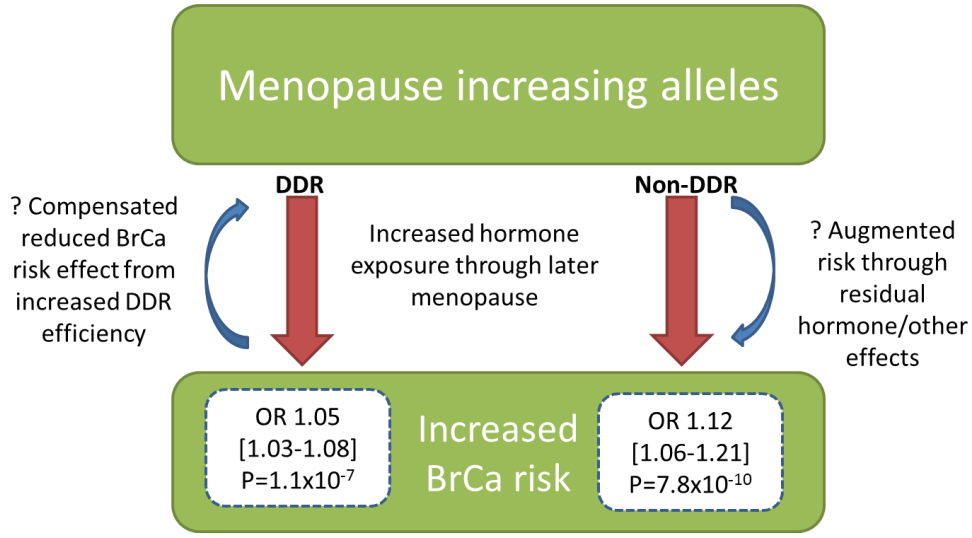




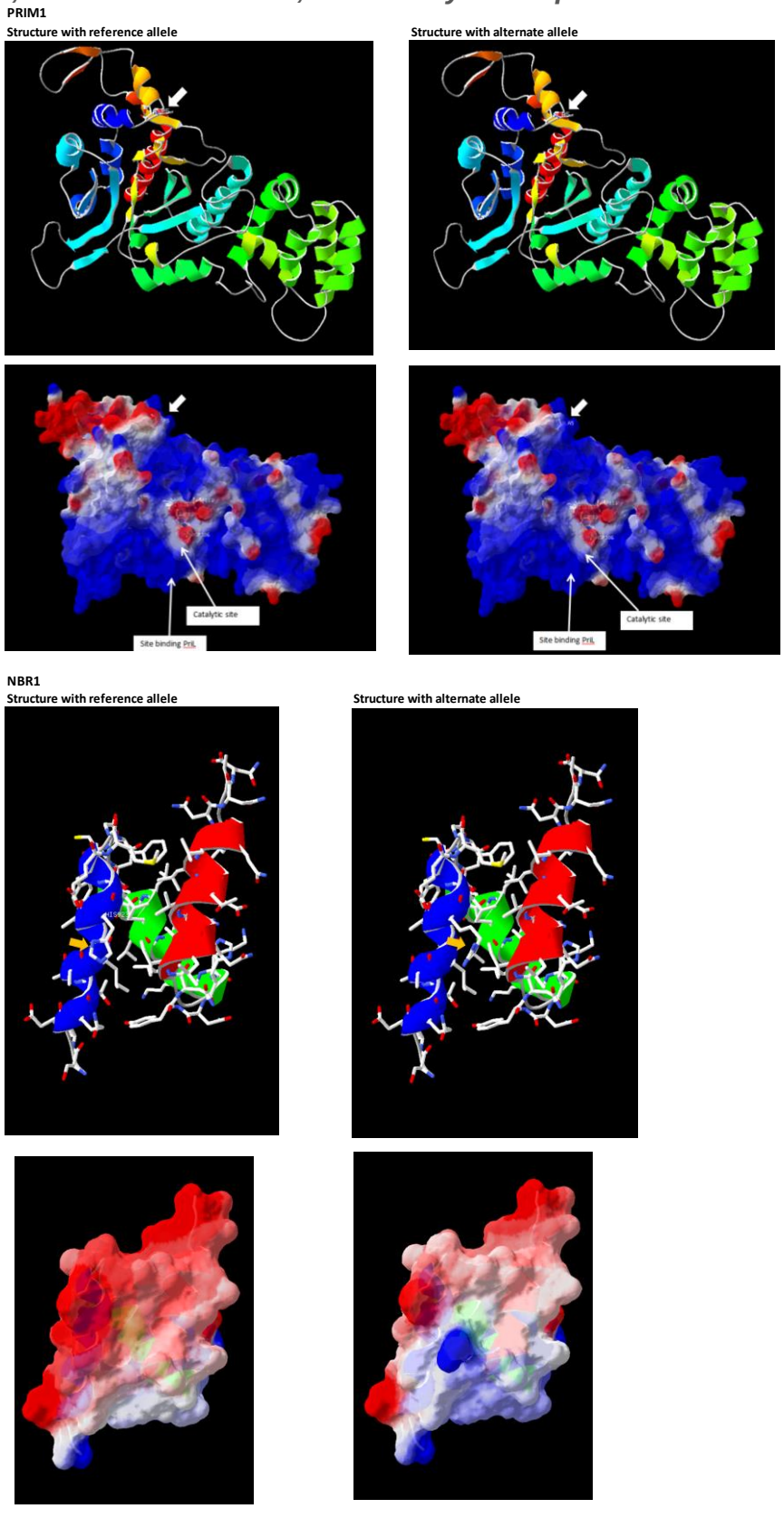




Supplementary Figure 4. Proposed mechanism of effect of SNPs on breast cancer risk.



Supplementary Figure 5. SWISS-MODEL predictions for two of the variants, in PRIM1 and NBR1, which may affect protein function.



Supplementary Tables

Table S1. Study level information for the contributing GWAS studies

Type	Study Name / acronym	Full Study name	Total N	Mean age collected (SD)	Mean age ANM (SD)	SNP array	Analysis program	Specific Menopause question
HM2 - old discovery	AGES	Age, Gene/ Environment Susceptibility Study	1315	76.34 (5.55)	48.9 (6.8)	Illumina HumanHap 370K CNV	PLINK	At what age did your menstrual periods permanently stop? Do not include menstrual period bleeding resulting from using female hormone pills. If you are not sure, please make your best guess. Ever had hysterectomy?
HM2 - old discovery	ARIC	Atherosclerosis Risk in Communities	2576	55.19 (5.40)	48.43 (4.03)	Affymetrix 6.0	ProbABEL	Have you reached menopause? Age when menopause began? Cause of menopause?
HM2 - old discovery	CHS	Cardiovascular Health Study	958	72.4 (5.5)	49.3 (4.3)	Illumina HumanHap 370K CNV	R-packages	How old were you at the time of your last natural period (menopause)?
HM2 - old discovery	deCODE		5857	birth year 1926.9 (8.9)	48.2(4.0)	Illumina HumanHap 300K and 370K CNV	Logstic regression using allele count as a covariate	How old were you when your menstruation ceased?
HM2 - old discovery	EGCUT (370k)	Estonian Genome Center, University of Tartu	279	61.1 (9.20)	49.5 (3.84)	Illumina HumanHap 370K CNV	SNPTEST	Have your periods stopped for 1 year or more? Age periods stopped? Cause periods stopped? Have you used hormonal medicaments due to menopause? When did you start using them?
HM2 - old discovery	ERF	Erasmus Rucphen Family study	373	47.83 (14.33)	49.35 (3.872)	Illumina 6K, Illumina 318K, Illumina 370K, Afyymetrix 250K	ProbABEL	Q1) At what age did the menstruation stopped (and began menopause)? Q2) Why did the menstruation stopped? Q3) Have you used medication (mostly HRT) due to menopause?
HM2 - old discovery	FHS	Framingham Heart Study	1452	NA	49.9 (3.51)	Affymetrix 500K + Affymetrix 50K	R-packages	Have your periods stopped for 1 year or more? Age periods stopped? Cause periods stopped?

Type	Study Name / acronym	Full Study name	Total N	Mean age collected (SD)	Mean age ANM (SD)	SNP array	Analysis program	Specific Menopause question
HM2 - old discovery	HAPI Heart Study	Heredity and Phenotype Intervention (HAPI) Heart Study	237	63.1 yrs (9.1)	49.0 (3.9)	Affymetrix 500K	MMAP	Have you reached menopause? Was your menopause natural or the result of surgery, radiation or chemotherapy? In what year or how old were you when you reached menopause?
HM2 - old discovery	InChianti	Invecchiare in Chianti	460	73.35 (8.65)	50.2 (4.09)	Illumina HumanHap 550K	SNPTEST	At what age did you go through the menopause.
HM2 - old discovery	NTR	Netherlands Twin Register	331	58.24 (5.96)	49.00 (3.69)	Affymetrix 500K Perlegen	SNPTEST	Have you reached the menopause (no menstrual period in the last 12 months)? Was this spontaneously? At what age did the menopause start?
HM2 - old discovery	QIMR	QIMR Berghofer	430	31.3 (10.3)	48.3 (4.4)	Illumina 317k / 370k / 610k	MERLIN -- fastassoc	Have your periods stopped for at least one year? If they have stopped, was this due to a) menopause b) hysterectomy c) other complications? What age were you when your periods stopped?
HM2 - old discovery	RSI	Rotterdam Study I	2196	70.35 (9.38)	49.87 (3.89)	Illumina HumanHap 550K	MACH2QTL	Did you have a menstrual period in the past 12 months? Age at last menstrual period? For what reasons did the periods stop?
HM2 - old discovery	RSII	Rotterdam Study II	665	65.65 (8.70)	50.52 (3.97)	Illumina HumanHap 550K	MACH2QTL	Did you have a menstrual period in the past 12 months? Age at last menstrual period? For what reasons did the periods stop?
HM2 - old discovery	RSIII	Rotterdam Study III	492	58.44 (5.45)	50.27 (3.83)	Illumina HumanHap 550K	MACH2QTL	Did you have a menstrual period in the past 12 months? Age at last menstrual period? For what reasons did the periods stop?
HM2 - old discovery	SardiNIA	SardiNIA	828	61.2 (10.12)	49.8 (4.13)	Affymetrix 500K + Affymetrix 10K	MERLIN -- fastassoc	How old were you at the time of your last natural period (menopause)? Cause periods stopped?
HM2 - old discovery	SHIP	Study of Health in Pomerania	405	55.0 (3.75)	49.7 (3.85)	Affymetrix 6.0	SNPTEST	How old have you been when your periods stopped? Have your periods stopped by natural menopause or due to operations or illness (natural/operation, illness/other)? Have you used hormones during your menopause or afterwards? (yes/no)
HM2 - old discovery	TwinsUKI		604	55.6 (6.70)	48.5(3.8)	Illumina HumanHap 300K	GenABEL	What was your age at last regular period?

Type	Study Name / acronym	Full Study name	Total N	Mean age collected (SD)	Mean age ANM (SD)	SNP array	Analysis program	Specific Menopause question
HM2 - old discovery	TwinsUKII		258	68.51(8.21)	47.67 (4.17)	Illumina Hap610Quad	GenABEL	What was your age at last regular period?
HM2 - old discovery	TwinsUKIII		743	67.51(7.87)	48.72 (4.33)	Illumina Hap610Quad	GenABEL	What was your age at last regular period?
HM2 - old discovery	WGHS	Women's Genome Health Study	11379	54.68 (7.19)	50.58 (3.64)	Illumina HumanHap300 Duo "+"	MACH2QTL	‘‘Have your menstrual periods ceased permanently?’’ If yes, ‘‘At what age did your natural periods cease?’’
HM2 - new discovery	GENOA	Genetic Epidemiology Network of Arteriopathy	301	62.74 (8.21)	50.09 (4.16)	Affymetrix 6.0 + Illumina 1M Duo	R-packages	In what year or how old were you when you reached menopause?
HM2 - new discovery	HRS	Health and Retirement Study	2546	69.72 (9.88)	50.60 (4.47)	Illumina Omni2.5	PLINK v1.07	About how old were you when you finished going through menopause, that is, had your last menstrual period?
HM2 - new discovery	HealthABC	The Health, Aging, and Body Composition Study	662	73.53 (2.82)	48.04 (4.78)	Illumina Human 1M-Duo	R-packages	How old were you at the time of your last natural menstrual period? Do not include menstrual bleeding due to taking female hormones.
HM2 - new discovery	MESA	Multi-Ethnic Study of Atherosclerosis	1098	64.43(9.37)	49.31(4.29)	Affymetrix 6.0	SNPTEST	Have you gone through menopause? At what age did you go through menopause?
HM2 - new discovery	KORA F3	Cooperative Health Research in the Region of Augsburg (follow-up 3)	391	65.4 (7.3)	50.2 (4.1)	Affymetrix 500k	SNPTEST v2.4.1	Did you have a menstrual period in the past 12 months? How old were you when you had your last menstrual period? Did you ever take hormones? How old were you then you took these hormones for the first time? How many months or years in total did you take these hormones? Do you currently take hormones? Did you have hysterectomy or ovarectomy?

Type	Study Name / acronym	Full Study name	Total N	Mean age collected (SD)	Mean age ANM (SD)	SNP array	Analysis program	Specific Menopause question
HM2 - new discovery	KORA F4	Cooperative Health Research in the Region of Augsburg (follow-up 4)	408	63.24 (7.38)	49.76 (3.96)	Affymetrix 6.0 (1000k)	SNPTEST v2.2.0	Did you have a menstrual period in the past 12 months? How old were you when you had your last menstrual period? Did you ever take hormone replacement therapy? How old were you then you took hormone replacement therapy for the first time? How many months or years in total did you take hormone replacement therapy? Do you currently take hormone replacement therapy? Did you have a hysterectomy or bilateral oophorectomy?
HM2 - new discovery	Lifelines	The LifeLInes Cohort Study and Biobank	1510	59.9 (7.6)	49.8 (4.2)	Illumina Cyto SNP12 v2	PLINK v1.07	At what age was your last period?
HM2 - new discovery	CROATIA-Korcula	CROATIA-Korcula	333	62.28(9.08)	49.6(4.12)	Illumina HumanHap 370CNV	GenABEL	How old were you when you had your last menstrual period?Are you having a regular menstrual cycle? Age at menopause? Have you had any type of surgery that could have induced menopause? Do you use HRT?
HM2 - new discovery	CROATIA-Split	CROATIA-Split	141	60.6(14.65)	49.99(3.82)	Illumina HumanHap370CNV	GenABEL	How old were you when you had your last menstrual period?Are you having a regular menstrual cycle? Age at menopause? Have you had any type of surgery that could have induced menopause? Do you use HRT?
HM2 - new discovery	CROATIA-Vis	CROATIA-Vis	313	65.65(15.54)	48.8(4.13)	Illumina HumanHap 300v1	GenABEL	How old were you when you had your last menstrual period?Are you having a regular menstrual cycle? Age at menopause? Have you had any type of surgery that could have induced menopause? Do you use HRT?
HM2 - new discovery	ORCADES	Orkney Complex Disease Study	145	62.46(7.81)	49.5(5.05)	Illumina HumanHap 300v1	GenABEL	Women were asked if they were still having periods. If they answered "no" they were asked their age.

Type	Study Name / acronym	Full Study name	Total N	Mean age collected (SD)	Mean age ANM (SD)	SNP array	Analysis program	Specific Menopause question
HM2 - new discovery	INGI-FVG	Genetic Park of Friuli Venezia Giulia Project	259	65.46 (9.6)	50.65 (3.70)	Illumina Infinium 370K	ProbABEL	Did you have a menstrual period in the past 12 months? Age at last menstrual period? Do you take hormones? Was your menopause natural, or the result of surgery, radiation, or chemotherapy? If yes, What age?
HM2 - new discovery	CARL	Cancer Aids Registries Linkage	125	62.39 (8.61)	49.02 (4.03)	Illumina Infinium 370K	ProbABEL	Did you have a menstrual period in the past 12 months? Age at last menstrual period? Do you take hormones? Was your menopause natural, or the result of surgery, radiation, or chemotherapy? If yes, What age?
HM2 - new discovery	Colaus	Etude Cohorte Lausannoise	1506	61.1 (7.2)	49.6 (4.1)	Affymetrix 500K	Matlab	At about what age was your last menstrual period? Did you already have a hysterectomy combined with an ovariectomy? Have you ever taken hormone replacement therapy (oestrogenes)?
HM2 - new discovery	INGI-VB	Val Borbera Isolated Population Project	523	66.8(10.7)	50.6(3.5)	Illumina 370k and Illumina 700k	GEMMA, GenABEL	Have you reached the menopause (no menstrual period in the last 12 months)? Was this spontaneously?
HM2 - new discovery	COGs	Breast Cancer Association Consortium	14884	62.22 (6.22)	50.36 (3.81)	Illuminia iselect "iCOGS"		Question varies by study
HM2 - new discovery	Generation Scotland	Generation Scotland:Scottish Family Health Study	882	60.43(8.08)	49.75(3.99)	Illumina OMNIexpress+exome	Probabel(1000G)	What age were you when you had your last period?
HM2 - new discovery	InterAct cohort	European Prospective Investigation into Cancer & Nutrition - InterAct	1761	60.03 (5.35)	49.23 (4.03)	Illumina 660 + Illumina Core+Exome	SNPTest	"If you are not still menstruating, how old were you when you stopped having your periods?"
HM2 - new discovery	InterAct cases	European Prospective Investigation into Cancer & Nutrition - InterAct	1386	59.27 (5.65)	49.26 (3.67)	Illumina 660 + Illumina Core+Exome	SNPTest	"If you are not still menstruating, how old were you when you stopped having your periods?"

Type	Study Name / acronym	Full Study name	Total N	Mean age collected (SD)	Mean age ANM (SD)	SNP array	Analysis program	Specific Menopause question
HM2 - new discovery	EGCUT OmniX	Estonian Genome Center, University of Tartu	1299	67.0 (9.6)	50.0 (3.8)	Illumina OmniExpress	SNPTESv1.1.5	Have your periods stopped for 1 year or more? Age periods stopped? Cause periods stopped? Have you used hormonal medicaments due to menopause? When did you start using them?
HM2 - new discovery	Cilento		291	49.50 (4.68)	49.94 (4.64)	Illumina 370 K and OmniExpress 700K	GenABEL and ProbABEL	Have you reached menopause? Age when menopause began? Cause of menopause?
HM2 - new discovery	NHS Illumina Chip	Nurses' Health Study	2482	54.25 (8.64)	50.63 (3.25)	Illumina 550k, 610Q, 660,660w, 300k, 610k	ProbABEL	1) Have your menstrual periods ceased permanently? Yes/no 2) If yes, for what reason? Surgery; radiation or chemotherapy;natural. 3) If yes, at what age?
HM2 - new discovery	NHS Omni Chip	Nurses' Health Study	2096	57.06 (6.49)	50.70 (3.25)	Omni Express	ProbABEL	1) Have your menstrual periods ceased permanently? Yes/no 2) If yes, for what reason? Surgery; radiation or chemotherapy;natural. 3) If yes, at what age?
HM2 - new discovery	NHS Affy Chip	Nurses' Health Study	2446	56.74 (6.70)	50.48 (3.30)	Affymetrix 6.0	ProbABEL	1) Have your menstrual periods ceased permanently? Yes/no 2) If yes, for what reason? Surgery; radiation or chemotherapy;natural. 3) If yes, at what age?

Table S2. The univariate results for the GWAS analysis, showing the nearest genes and genes within 500kb.

SNP	Chr	Position	P-value	Chr. band	Closest Gene	Distance to closest gene (bp)	Other genes within 500kb
rs4246511	1	39152972	5.09E-21	p34.3	*RHBDL2*	0	*RRAGC, GJA9-MYCBP, MYCBP, GJA9, RHBDL2, AKIRIN1, NDUFS5, MACF1, KIAA0754*
rs12142240	1	46519888	6.62E-09	p33	*LRRC41*	0	*MAST2, PIK3R3, TSPAN1, POMGNT1, LURAP1, RAD54L, LRRC41, UQCRH, NSUN4, FAAH, LOC729041, DMBX1, LOC100507423, KNCN, MKNK1, MOB3C, ATPAF1, ATPAF1-AS1, LOC100130197, KIAA0494*
rs1411478	1	179228905	1.35E-10	q25.3	*STX6*	0	*ACBD6, XPR1, KIAA1614, STX6, MR1, IER5, GM140, CACNA1E*
rs2236918	1	240084449	8.31E-14	q43	*EXO1*	0	*RGS7, FH, KMO, OPN3, CHML, WDR64, EXO1, MAP1LC3C, PLD5*
rs704795	2	27569998	2.08E-15	p23.3	*FNDC4*	0	*MAPRE3, TMEM214, AGBL5, OST4, EMILIN1, KHK, CGREF1, ABHD1, PREB, C2orf53, TCF23, SLC5A6, C2orf28, CAD, SLC30A3, DNAJC5G, TRIM54, UCN, MPV17, GTF3C2, LOC100505624, EIF2B4, SNX17, ZNF513, PPM1G, FTH1P3, NRBP1, KRTCAP3, IFT172, FNDC4, GCKR, C2orf16, ZNF512, CCDC121, GPN1, SUPT7L, SLC4A1AP, MRPL33, RBKS, LOC100302650, BRE*
rs1800932	2	47871585	3.18E-11	p16.3	*MSH6*	0	*EPCAM, MIR559, MSH2, KCNK12, MSH6, FBXO11*
rs7598194	2	171479657	6.21E-12	q31.1	*GORASP2*	13536	*MYO3B, LOC440925, SP5, LOC100505695, GAD1, GORASP2, TLK1, METTL8*
rs930036	2	171649264	3.07E-19	q31.1	*TLK1*	0	*MYO3B, LOC440925, SP5, LOC100505695, GAD1, GORASP2, TLK1, METTL8, DCAF17, CYBRD1*
rs7573003	2	172449661	3.93E-08	q31.1	*SLC25A12*	0	*METTL8, DCAF17, CYBRD1, DYNC1I2, SLC25A12, HAT1, METAP1D, DLX1, DLX2*
rs16858210	3	185106704	3.07E-09	q27.1	*ABCC5*	13713	*MCF2L2, LOC100505687, KLHL6, KLHL24, YEATS2, MAP6D1, PARL, ABCC5, HTR3D, HTR3C, HTR3E, EIF2B5, DVL3, AP2M1, ABCF3, VWA5B2, MIR1224, ALG3, ECE2, CAMK2N2, PSMD2, EIF4G1, SNORD66, FAM131A, CLCN2, POLR2H, THPO, CHRD*
rs4693089	4	84592646	9.16E-23	q21.23	*HELQ*	0	*LIN54, COPS4, PLAC8, COQ2, HPSE, HELQ, MRPS18C, FAM175A, AGPAT9*

SNP	Chr	Position	P-value	Chr. band	Closest Gene	Distance to closest gene (bp)	Other genes within 500kb
rs6856693	4	185985800	9.79E-15	q35.1	*ACSL1*	1591	*LOC728175, IRF2, CASP3, CCDC111, MLF1IP, ACSL1, SLED1, LOC731424, MIR3945, LOC100506229, HELT, SLC25A4, KIAA1430, SNX25*
rs427394	5	6798875	3.81E-09	p15.31	*PAPD7*	0	*FLJ33360, MED10, UBE2QL1, LOC255167, NSUN2, SRD5A1, PAPD7, MIR4278*
rs11738223	5	171867097	1.96E-08	q35.1	*SH3PXD2B*	52965	*STK10, EFCAB9, UBTD2, SH3PXD2B, NEURL1B, DUSP1, ERGIC1, LOC100268168, RPL26L1, ATP6V0E1*
rs365132	5	176311180	1.41E-33	q35.2	*UIMC1*	0	*FAF2, RNF44, CDHR2, GPRIN1, SNCB, MIR4281, EIF4E1B, TSPAN17, UNC5A, HK3, UIMC1, ZNF346, FGFR4, NSD1, RAB24, PRELID1, MXD3, LMAN2, RGS14, SLC34A1, PFN3, F12, GRK6, LOC340037, PRR7, PRR7*
rs6899676	6	11003246	2.22E-19	p24.2	*SYCP2L*	0	*TFAP2A, LOC100130275, LINC00518, GCNT2, C6orf52, PAK1IP1, TMEM14C, TMEM14B, MAK, GCM2, SYCP2L, ELOVL2, LOC100506409, C6orf228, ERVFRD-1, NEDD9, NEDD9*
rs3094222	6	31189413	7.79E-09	p21.33	*C6orf15*	1102	*PPP1R10, MRPS18B, ATAT1, C6orf136, DHX16, PPP1R18, NRM, MDC1, TUBB, FLOT1, IER3, DDR1, MIR4640, GTF2H4, VARS2, SFTA2, DPCR1, MUC21, MUC22, HCG22, C6orf15, PSORS1C1, CDSN, PSORS1C2, CCHCR1, TCF19, POU5F1, PSORS1C3, HCG27, HLA-C, HLA-B, MICA, HCP5, HCG26, MICB, MCCD1, DDX39B, ATP6V1G2-DDX39B, SNORD117, SNORD84, ATP6V1G2, NFKBIL1, LTA, TNF, LTB, LST1, NCR3*

SNP	Chr	Position	P-value	Chr. band	Closest Gene	Distance to closest gene (bp)	Other genes within 500kb
rs1046089	6	31710946	3.66E-21	p21.33	*PRRC2A*	0	*PSORS1C1, PSORS1C2, CCHCR1, TCF19, POU5F1, PSORS1C3, HCG27, HLA-C, HLA-B, MICA, HCP5, HCG26, MICB, MCCD1, DDX39B, ATP6V1G2-DDX39B, SNORD117, SNORD84, ATP6V1G2, NFKBIL1, LTA, TNF, LTB, LST1, NCR3, AIF1, PRRC2A, SNORA38, BAG6, APOM, C6orf47, GPANK1, CSNK2B, LY6G5B, LY6G5C, ABHD16A, MIR4646, LY6G6F, LY6G6E, LY6G6D, LY6G6C, C6orf25, DDAH2, CLIC1, MSH5, MSH5-SAPCD1, SAPCD1, VWA7, VARS, LSM2, HSPA1L, HSPA1A, HSPA1B, C6orf48, SNORD48, SNORD52, NEU1, SLC44A4, EHMT2, ZBTB12, C2, CFB, RDBP, MIR1236, SKIV2L, DOM3Z, STK19, LOC100293534, C4B, C4A, CYP21A1P, TNXA, STK19, C4B, LOC100293534, C4A, CYP21A2, TNXB, ATF6B, FKBPL*
rs3134942	6	32276749	2.91E-09	p21.32	*NOTCH4*	0	*ABHD16A, MIR4646, LY6G6F, LY6G6E, LY6G6D, LY6G6C, C6orf25, DDAH2, CLIC1, MSH5, MSH5-SAPCD1, SAPCD1, VWA7, VARS, LSM2, HSPA1L, HSPA1A, HSPA1B, C6orf48, SNORD48, SNORD52, NEU1, SLC44A4, EHMT2, ZBTB12, C2, CFB, RDBP, MIR1236, SKIV2L, DOM3Z, STK19, LOC100293534, C4B, C4A, CYP21A1P, TNXA, STK19, C4B, LOC100293534, C4A, CYP21A2, TNXB, ATF6B, FKBPL, PRRT1, LOC100507547, PPT2, PPT2-EGFL8, EGFL8, AGPAT1, RNF5, RNF5P1, AGER, PBX2, GPSM3, NOTCH4, C6orf10, HCG23, BTNL2, HLA-DRA, HLA-DRB5, HLA-DRB6, HLA-DRB1, HLA-DQA1, HLA-DQB1*
rs12196873	6	111704751	2.79E-08	q21	*KIAA1919*	7797	*CDK19, AMD1, GTF3C6, RPF2, GSTM2P1, SLC16A10, KIAA1919, REV3L, TRAF3IP2-AS1, TRAF3IP2, FYN*
rs2720044	8	38099744	7.33E-22	p12	*ASH2L*	0	*ZNF703, ERLIN2, ERLIN2, LOC728024, PROSC, GPR124, BRF2, RAB11FIP1, GOT1L1, ADRB3, EIF4EBP1, ASH2L, STAR, LSM1, BAG4, DDHD2, PPAPDC1B, WHSC1L1, LETM2, FGFR1, C8orf86, RNF5P1*

SNP	Chr	Position	P-value	Chr. band	Closest Gene	Distance to closest gene (bp)	Other genes within 500kb
rs10957156	8	61791955	4.49E-09	q12.2	*CHD7*	0	*CA8, RAB2A, CHD7, LOC100130298*
rs4879656	9	33002382	2.04E-08	p13.3	*APTX*	10743	*DDX58, TOPORS, LOC100129250, NDUFB6, TAF1L, TMEM215, APTX, DNAJA1, SMU1, B4GALT1, SPINK4, BAG1, CHMP5, NFX1, AQP7, AQP3, NOL6, SUGT1P1*
rs10905065	10	5809833	3.93E-08	p15.1	*FAM208B*	0	*UCN3, TUBAL3, NET1, CALML5, CALML3, ASB13, FAM208B, GDI2, ANKRD16, FBXO18, IL15RA, IL2RA, RBM17, PFKFB3, MIR3155A, MIR3155B*
rs11031006	11	30183104	8.49E-14	p14.1	*FSHB*	26034	*KCNA4, FSHB, C11orf46, MPPED2, MPPED2*
rs10734411	11	32498360	2.59E-09	p13	*EIF3M*	63528	*RCN1, WT1, WT1-AS, EIF3M, CCDC73, PRRG4, QSER1, DEPDC7*
rs2277339	12	55432336	1.78E-19	q13.3	*PRIM1*	0	*ANKRD52, COQ10A, CS, CNPY2, PAN2, IL23A, STAT2, APOF, TIMELESS, MIP, SPRYD4, GLS2, RBMS2, BAZ2A, ATP5B, SNORD59B, SNORD59A, PTGES3, NACA, PRIM1, HSD17B6, SDR9C7, RDH16, GPR182, ZBTB39, TAC3, MYO1A, TMEM194A, NAB2, STAT6, LRP1, MIR1228, NXPH4, SHMT2, NDUFA4L2, STAC3*
rs12371165	12	65101815	7.00E-10	q14.3	*GRIP1*	0	*HMGA2, LLPH, TMBIM4, IRAK3, HELB, GRIP1*
rs551087	12	119693576	3.93E-08	q24.31	*SPPL3*	0	*SIRT4, PLA2G1B, MSI1, COX6A1, TRIAP1, GATC, SRSF9, DYNLL1, LOC100506668, COQ5, RNF10, POP5, CABP1, MLEC, UNC119B, MIR4700, ACADS, SPPL3, HNF1A-AS1, HNF1A, C12orf43, OASL, P2RX7, P2RX4, CAMKK2*
rs1727326	12	122166039	1.66E-09	q24.31	*PITPNM2*	5111	*KNTC1, HCAR2, HCAR3, HCAR1, DENR, CCDC62, HIP1R, VPS37B, ABCB9, OGFOD2, ARL6IP4, PITPNM2, MIR4304, LOC100507091, MPHOSPH9, C12orf65, CDK2AP1, SBNO1, SETD8, RILPL2, SNRNP35, RILPL1, MIR3908, TMED2, DDX55*
rs12824058	12	129370287	6.13E-11	q24.33	*PIWIL1*	18098	*TMEM132D, LOC100190940, FLJ31485, FZD10, PIWIL1, RIMBP2, STX2*
rs4886238	13	60011740	2.50E-16	q21.2	*TDRD3*	0	*DIAPH3, DIAPH3, TDRD3*

SNP	Chr	Position	P-value	Chr. band	Closest Gene	Distance to closest gene (bp)	Other genes within 500kb
rs1713460	14	20003455	2.35E-10	q11.2	*PNP*	3922	*OR4K15, OR4K14, OR4K13, OR4L1, OR4K17, OR4N5, OR11G2, OR11H6, OR11H4, TTC5, CCNB1IP1, SNORD126, RPPH1, PARP2, TEP1, KLHL33, OSGEP, APEX1, TMEM55B, PNP, RNASE10, RNASE9, RNASE11, RNASE12, OR6S1, ANG, RNASE4, EDDM3A, EDDM3B, RNASE6, RNASE1, RNASE3, ECRP, RNASE2*
rs9796	15	39058739	1.31E-10	q15.1	*INO80*	0	*MRPL42P5, C15orf57, RPUSD2, CASC5, LOC100505648, RAD51, FAM82A2, GCHFR, DNAJC17, C15orf62, ZFYVE19, PPP1R14D, SPINT1, RHOV, VPS18, DLL4, CHAC1, INO80, EXD1, CHP, OIP5-AS1, OIP5, NUSAP1, NDUFAF1, RTF1*
rs1054875	15	87680130	1.65E-19	q26.1	*POLG*	1100	*ACAN, HAPLN3, MFGE8, ABHD2, RLBP1, FANCI, POLG, MIR9-3, LOC254559, RHCG, LOC283761, C15orf42, KIF7, PLIN1, PEX11A, WDR93, MESP1, MESP2, ANPEP, C15orf38-AP3S2, AP3S2*
rs9039	16	9112864	3.26E-08	p13.2	*C16orf72*	0	*METTL22, ABAT, TMEM186, PMM2, CARHSP1, USP7, C16orf72, MIR548X*
rs10852344	16	11924420	1.30E-15	p13.13	*GSPT1*	6400	*LITAF, SNN, TXNDC11, ZC3H7A, BCAR4, RSL1D1, GSPT1, TNFRSF17, SNX29*
rs12599106	16	34355526	3.13E-08	p11.2	*UBE2MP1*	93263	*LINC00273, UBE2MP1, LOC283914, LOC283914, LOC146481, LOC100130700, FLJ26245*
rs8070740	17	5272620	1.53E-09	p13.2	*RPAIN*	0	*GP1BA, SLC25A11, RNF167, PFN1, ENO3, SPAG7, CAMTA2, INCA1, KIF1C, SLC52A1, ZFP3, ZNF232, USP6, ZNF594, LOC100130950, SCIMP, RABEP1, NUP88, RPAIN, C1QBP, DHX33, DERL2, MIS12, LOC728392, NLRP1, LOC339166*
rs2941505	17	35086230	1.94E-09	q12	*PGAP3*	0	*CACNB1, RPL19, STAC2, FBXL20, MED1, CDK12, NEUROD2, PPP1R1B, STARD3, TCAP, PNMT, PGAP3, ERBB2, MIR4728, MIEN1, GRB7, IKZF3, ZPBP2, GSDMB, ORMDL3, LRRC3C, GSDMA, PSMD3, CSF3, MED24, SNORD124, THRA, THRA, NR1D1, MSL1, CASC3*

SNP	Chr	Position	P-value	Chr. band	Closest Gene	Distance to closest gene (bp)	Other genes within 500kb
rs1799949	17	38498992	8.39E-11	q21.31	*BRCA1*	0	*FAM134C, TUBG1, TUBG2, PLEKHH3, CCR10, CNTNAP1, EZH1, LOC100190938, RAMP2, VPS25, WNK4, CCDC56, CNTD1, BECN1, PSME3, AOC2, AOC3, AOC4, LOC388387, G6PC, AARSD1, RUNDC1, RPL27, IFI35, VAT1, RND2, BRCA1, NBR2, NBR1, TMEM106A, LOC100130581, ARL4D, MIR2117, DHX8, ETV4*
rs349306	19	901694	1.72E-10	p13.3	*ARID3A*	0	*SHC2, ODF3L2, MADCAM1, TPGS1, CDC34, GZMM, BSG, HCN2, POLRMT, FGF22, RNF126, FSTL3, PRSS57, PALM, C19orf21, PTBP1, MIR4745, LPPR3, MIR3187, AZU1, PRTN3, ELANE, CFD, MED16, R3HDM4, KISS1R, ARID3A, WDR18, GRIN3B, C19orf6, CNN2, ABCA7, HMHA1, POLR2E, GPX4, SBNO2, STK11, C19orf26, ATP5D, MIDN, CIRBP-AS1, CIRBP, C19orf24, EFNA2, MUM1, NDUFS7, GAMT, DAZAP1, RPS15, APC2*
rs7259376	19	22299545	4.18E-08	p12	*ZNF729*	7727	*ZNF43, ZNF208, ZNF257, ZNF676, ZNF729, ZNF98, LOC440518, ZNF492, ZNF99*
rs11668344	19	60525476	5.51E-85	q13.42	*TMEM150B*	0	*KIR3DL1, KIR2DS4, KIR3DL2, FCAR, NCR1, NLRP7, NLRP2, GP6, RDH13, EPS8L1, PPP1R12C, TNNT1, TNNI3, DNAAF3, SYT5, PTPRH, TMEM86B, PPP6R1, HSPBP1, BRSK1, TMEM150B, SUV420H2, COX6B2, FAM71E2, IL11, TMEM190, TMEM238, RPL28, UBE2S, SHISA7, ISOC2, ZNF628, NAT14, SSC5D, SBK2, SGK110, ZNF579, FIZ1, ZNF524, ZNF865, ZNF784, ZNF580, ZNF581, CCDC106, U2AF2, EPN1, NLRP9, RFPL4A, NLRP11*
rs16991615	20	5896227	1.58E-89	p12.3	*MCM8*	0	*LOC643406, LOC149837, GPCPD1, C20orf196, CHGB, TRMT6, MCM8, CRLS1, LRRN4, FERMT1*
rs13040088	20	61019647	2.45E-10	q13.33	*DIDO1*	0	*C20orf166-AS1, C20orf166, MIR1-1, MIR133A2, SLCO4A1, LOC100127888, NTSR1, LOC100652730, C20orf20, OGFR, COL9A3, TCFL5, DPH3P1, DIDO1, C20orf11, SLC17A9, BHLHE23, LOC63930, LINC00029, LOC100144597, HAR1B, HAR1A, MIR124-3, YTHDF1, BIRC7, MIR3196, NKAIN4, FLJ16779, ARFGAP1, MIR4326, COL20A1, CHRNA4, KCNQ2*

SNP	Chr	Position	P-value	Chr. band	Closest Gene	Distance to closest gene (bp)	Other genes within 500kb
rs5762534	22	26963571	6.12E-09	q12.1	*MIR548AM*	0	*MN1, MIR548AM, PITPNB, TTC28-AS1, MIR3199-1, MIR3199-2, TTC28, CHEK2*
rs763121	22	37209886	2.27E-13	q13.1	*DDX17*	0	*SOX10, MIR4534, PICK1, SLC16A8, BAIAP2L2, PLA2G6, MAFF, TMEM184B, CSNK1E, LOC400927, KCNJ4, KDELR3, DDX17, DMC1, LOC646851, CBY1, TOMM22, JOSD1, GTPBP1, SUN2, DNAL4, NPTXR, CBX6, APOBEC3A, APOBEC3B*

Table S3. The results from GCTA showing secondary signals, and also the highlighted genes from the pathway analysis.

						Univariate Model		Joint Model		Highlighted gene	GRAIL gene	Muther eQTL	Blood eQTL	High LD Coding	Chr	Closest
Best SNP	**Signal SNP**	**Chr**	**position**	**Alleles**	**N**	**Effect**	**P**	**Effect**	**P**	**(DDR genes in bold)**					**region**	**gene**
rs4246511	rs4246511	1	39,152,972	c/t/0.71	69116	-0.22 (0.02)	5.1E-21	-	-	*RHBDL2(B,N) / **MYCBP** (B)*	RHBDL2 / LRRC41				p34.3	*RHBDL2*
rs12142240	rs12142240	1	46,519,888	t/c/0.68	69356	-0.13 (0.02)	6.6E-09	-	-	***RAD54L** (B,E)*	RAD54L	*FAAH*	NSUN4		p33	*LRRC41*
rs1411478	rs1411478	1	179,228,905	a/g/0.41	68680	-0.13 (0.02)	1.4E-10	-	-	*STX6 (N,E)*					q25.3	*STX6*
rs2236918	rs2236918	1	240,084,449	c/g/0.45	69332	-0.15 (0.02)	8.3E-14	-	-	***EXO1** (N,B,C)*	EXO1			*EXO1*	q43	*EXO1*
rs704795	rs704795	2	27,569,998	a/g/0.4	69341	-0.16 (0.02)	2.1E-15	-	-	***BRE** (B) / GTF3C2 (B,E)/ EIFB4 (B)*		*GTF3C2*	KRTCAP3; IFT172		p23.3	*FNDC4*
rs1800932	rs1800932	2	47,871,585	a/g/0.81	69309	-0.17 (0.03)	3.2E-11	-	-	***MSH6** (N,B,E)*	MSH6				p16.3	*MSH6*
rs930036	rs930036	2	171,649,264	a/g/0.38	69357	-0.19 (0.02)	3.1E-19	-	-	***TLK1** (N,E,B) / GAD1 (B)*					q31.1	*TLK1*
rs16858210	rs16858210	3	185,106,704	g/a/0.75	69193	-0.14 (0.02)	3.1E-09	-	-	***PARL** (B) / **POLR2H** (B)*	PARL				q27.1	*ABCC5*
rs4693089	rs4693089	4	84,592,646	a/g/0.51	69060	-0.2 (0.02)	9.2E-23	-	-	***HELQ** (N,B) / **FAM175A** (B)*					q21.23	*HELQ*
rs6856693	rs6856693	4	185,985,800	a/g/0.58	67635	-0.16 (0.02)	9.8E-15	-	-	*ASCL1 (N), **MLF1IP** (B)*					q35.1	*ACSL1*
rs427394	rs427394	5	6,798,875	g/a/0.41	69284	-0.13 (0.02)	3.8E-09	-	-	***PAPD7** (N,B)*	POLS				p15.31	*PAPD7*
rs11738223	rs11738223	5	171,867,097	a/g/0.68	69250	-0.12 (0.02)	2.0E-08	-	-	*SH3PXD2B (N)*					q35.1	*SH3PXD2B*
rs365132	rs2241584	5	175,888,783	a/g/0.38	69341	-0.14 (0.02)	1.5E-11	-0.14 (0.02)	3.2E-11	***UIMC1** (B,E)*		*ZNF346*	UIMC1		q35.2	*RNF44*
"	rs365132	5	176,311,180	g/t/0.51	69349	-0.24 (0.02)	1.4E-33	-0.24 (0.02)	7.9E-33	***UIMC1** (N,B,E)*					q35.2	*UIMC1*
rs6899676	rs6899676	6	11,003,246	a/g/0.8	69303	-0.23 (0.03)	2.2E-19	-0.21 (0.03)	6.2E-16	*SYCP2L (N,B) / MAK (B)*					p24.2	*SYCP2L*
"	rs9393800	6	11,059,723	g/a/0.27	69124	-0.17 (0.02)	3.5E-13	-0.14 (0.02)	1.1E-09	*SYCP2L (N,B) / MAK (B)*					p24.2	*SYCP2L*
rs1046089	rs2230365	6	31,633,427	c/t/0.84	67095	-0.17 (0.03)	7.6E-10	-0.16 (0.03)	2.7E-08	***MSH5** (B) / HLA (B)*	*MSH5*				p21.33	*NFKBIL1*
"	rs707938	6	31,837,338	g/a/0.32	68582	-0.17 (0.02)	7.2E-15	-0.16 (0.02)	2.3E-13	***MSH5** (B,N, E) / HLA (B)*	*MSH5*		SNORD52; SNORD48; C6orf48		p21.33	*MSH5*
rs12196873	rs12196873	6	111,704,751	a/c/0.85	69313	-0.16 (0.03)	2.8E-08	-	-	***REV3L** (B,C)*	*REV3L*			*REV3L*	q21	*KIAA1919*
rs2720044	rs2720044	8	38,099,744	a/c/0.84	63917	-0.29 (0.03)	7.3E-22	-	-	*STAR (B)*	*STAR*				p12	*ASH2L*
rs10957156	rs10957156	8	61,791,955	a/g/0.76	69341	-0.14 (0.02)	4.5E-09	-	-	***CHD7** (N,B,E)*					q12.2	*CHD7*
rs4879656	rs4879656	9	33,002,382	a/c/0.37	68919	-0.12 (0.02)	2.0E-08	-	-	***APTX** (N,B,E)*		APTX / SMU1	APTX		p13.3	*APTX*
rs10905065	rs10905065	10	5,809,833	a/g/0.61	69334	-0.11 (0.02)	3.9E-08	-	-	***FBXO18** (B)*					p15.1	*FAM208B*
rs11031006	rs11031006	11	30,183,104	g/a/0.85	69309	-0.22 (0.03)	8.5E-14	-0.25 (0.03)	4.0E-17	*FSHB (N,B)*					p14.1	*FSHB*
"	rs6484478	11	30,263,016	g/a/0.74	69099	-0.1 (0.02)	4.0E-05	-0.14 (0.02)	1.0E-08	*FSHB (B)*					p14.1	*C11orf46*
rs10734411	rs10734411	11	32,498,360	a/g/0.47	69142	-0.12 (0.02)	2.6E-09	-	-	*EIF3M (N)*					p13	*EIF3M*
rs2277339	rs2277339	12	55,432,336	g/t/0.1	67603	-0.31 (0.03)	1.8E-19	-	-	***PRIM1** (B,N,C,E) / TAC3 (B)*			PRIM1	*PRIM1*	q13.3	*PRIM1*

							Univariate Model		Joint Model		Highlighted gene	GRAIL gene	Muther eQTL	Blood eQTL	High LD Coding	Chr	Closest
Region	Best SNP	Signal SNP	Chr	position	Alleles	N	Effect	P	Effect	P	(DDR genes in bold)					region	gene
24a	rs12371165	rs3741604	12	64,982,677	t/c/0.52	69100	-0.09 (0.02)	1.9E-05	-0.29 (0.03)	1.8E-21	***HELB*** *(N,B,E,C)*		HELB		*HELB*	q14.3	*HELB*
24b	"	rs1183272	12	65,021,688	c/t/0.45	68727	-0.07 (0.02)	7.3E-04	-0.31 (0.03)	3.0E-24	***HELB*** *(B,N,C)*				*HELB*	q14.3	*HELB*
24c	"	rs7397861	12	65,100,733	g/c/0.64	69095	-0.1 (0.02)	6.7E-06	-0.13 (0.02)	4.6E-09	***HELB*** *(B,E,C)*			HELB		q14.3	*GRIP1*
25	rs551087	rs551087	12	119,693,576	g/a/0.29	69001	-0.13 (0.02)	3.9E-08	-	-	*SPPL3 (N) / SRSF9 (B)*					q24.31	*SPPL3*
26	rs1727326	rs1727326	12	122,166,039	c/g/0.15	68870	-0.19 (0.03)	1.7E-09	-	-	***KNTC1*** *(B), PITPNM2 (N)*					q24.31	*PITPNM2*
27	rs12824058	rs12824058	12	129,370,287	g/a/0.43	69047	-0.14 (0.02)	6.1E-11	-	-	*PIWIL1 (N)*					q24.33	*PIWIL1*
28	rs4886238	rs4886238	13	60,011,740	g/a/0.66	69314	-0.18 (0.02)	2.5E-16	-	-	*TDRD3 (B,N)*					q21.2	*TDRD3*
29	rs1713460	rs1713460	14	20,003,455	g/a/0.3	68528	-0.14 (0.02)	2.4E-10	-	-	***APEX1*** *(B) /* ***PARP2*** *(B) / PNP (N,E)*	*APEX1*				q11.2	*PNP*
30	rs9796	rs9796	15	39,058,739	t/a/0.46	69317	-0.13 (0.02)	1.3E-10	-	-	***INO80*** *(B,N,E) /* ***RAD51*** *(B)*					q15.1	*INO80*
31	rs1054875	rs1054875	15	87,680,130	t/a/0.4	69288	-0.19 (0.02)	1.7E-19	-	-	***POLG*** *(B,N) /* ***FANCI*** *(B,C)*	*POLG*			*FANCI*	q26.1	*POLG*
32	rs9039	rs9039	16	9,112,864	c/t/0.28	69341	-0.12 (0.02)	3.3E-08	-	-	*C16orf72 (N) / ABAT (B)*					p13.2	*C16orf72*
33	rs10852344	rs10852344	16	11,924,420	t/c/0.59	69346	-0.16 (0.02)	1.3E-15	-	-	*GSPT1 (N,C,E) / BCAR4 (B)*				*GSPT1*	p13.13	*GSPT1*
34	rs12599106	rs12599106	16	34,355,526	a/t/0.51	69320	-0.12 (0.02)	3.1E-08	-	-	*UBE2MP1 (N)*					p11.2	*UBE2MP1*
35	rs8070740	rs8070740	17	5,272,620	a/g/0.76	68515	-0.15 (0.02)	1.5E-09	-	-	*RPAIN (N,E)*			RPAIN		p13.2	*RPAIN*
36	rs2941505	rs2941505	17	35,086,230	a/g/0.32	69302	-0.13 (0.02)	1.9E-09	-	-	*STARD3 (B) / PGAP3 (N,E) / CDK12 (B)*	*STARD3*	PGAP3	PGAP3		q12	*PGAP3*
37	rs1799949	rs1799949	17	38,498,992	g/a/0.68	69329	-0.14 (0.02)	8.4E-11	-	-	***BRCA1*** *(N,E,B,C)*		BRCA1 / RND2 / VAT1	BRCA1 / VAT1	*BRCA1*	q21.31	*BRCA1*
38	rs349306	rs349306	19	901,694	g/a/0.13	58278	-0.23 (0.04)	1.7E-10	-	-	***POLR2E*** *(B) / KISS1R (B)*					p13.3	*ARID3A*
39	rs7259376	rs7259376	19	22,299,545	a/g/0.46	69328	-0.11 (0.02)	4.2E-08	-	-	*ZNF729 (N)*					p12	*ZNF729*
40a	rs11668344	rs11668344	19	60,525,476	g/a/0.36	69329	-0.41 (0.02)	5.5E-85	-0.41 (0.02)	4.2E-84	***BRSK1*** *(B,E) / NLRP11 (N) / U2AF2 (B)*		BRSK1			q13.42	*TMEM150B*
40b	"	rs2547274	19	61,002,040	g/c/0.91	66580	-0.28 (0.04)	3.4E-13	-0.22 (0.04)	2.7E-08	***BRSK1*** *(B) / NLRP11 (N) / U2AF2 (B)*					q13.42	*NLRP11*
40c	"	rs12461110	19	61,012,475	a/g/0.35	68518	-0.17 (0.02)	7.6E-16	-0.15 (0.02)	5.0E-12	***BRSK1*** *(B) / NLRP11 (N,C) / U2AF2 (B)*				*NLRP11*	q13.42	*NLRP11*
41a	rs16991615	rs451417	20	5,889,999	a/c/0.12	65420	-0.2 (0.03)	4.6E-09	-0.2 (0.03)	4.5E-09	***MCM8*** *(N,C, B)*				*MCM8*	p12.3	*MCM8*
41b	"	rs16991615	20	5,896,227	g/a/0.93	66210	-0.88 (0.04)	1.6E-89	-0.88 (0.04)	4.4E-89	***MCM8*** *(N,C, B)*				*MCM8*	p12.3	*MCM8*
42a	rs13040088	rs2236553	20	60,760,188	c/t/0.24	62648	-0.16 (0.03)	6.1E-10	-0.16 (0.03)	4.4E-10	*SLCO4A1 (N,C) /* ***DIDO1*** *(B,E)*			DIDO1	*DIDO1*	q13.33	*SLCO4A1*
42b	"	rs13040088	20	61,019,647	g/a/0.21	69317	-0.16 (0.02)	2.4E-10	-0.16 (0.02)	1.9E-10	*SLCO4A1 (C) /* ***DIDO1*** *(N,B,E)*					q13.33	*DIDO1*
43	rs5762534	rs5762534	22	26,963,571	t/c/0.84	69322	-0.16 (0.03)	6.1E-09	-	-	***CHEK2*** *(B)*	*CHEK2*				q12.1	*MIR548AM*
44	rs763121	rs763121	22	37,209,886	g/a/0.36	66632	-0.16 (0.02)	2.3E-13	-	-	***DMC1*** *(B) / DDX17 (N,E,B)*	*DMC1*			*KDELR3*	q13.1	*DDX17*

Table S4. Variance explained estimates from GCTA using the InterAct cohort data.

Cut Off for SNP set	Number of SNPs	Variance Estimate	SE	p
All QD'd (unclumped)	2,797,986	0.3407	0.1789	0.02676
0.05	29,958	0.2099	0.0974	0.01261
0.005	5,602	0.1717	0.0466	6.47E-05
5.00E-04	1,070	0.0848	0.0242	4.16E-05
5.00E-05	378	0.0825	0.0186	1.33E-09
5.00E-06	208	0.0785	0.0177	2.50E-12
5.00E-07	128	0.0604	0.0161	2.06E-11
GWAS sigificant	54	0.0567	0.0161	6.26E-12

Table S5. Partitioning heritability. Results of 10 tissue categories from Broad analysis.

(Note: Most significant results shown. For full table see Supplementary in published paper.)

Name	Mark	Prop._SNPs	Prop._h2	Prop._h2_std_error	Prop. h2 p	Enrichment	Enrichment_std_error	Enrichment p	Coefficient	Coefficient_std_error	Coefficient_z-score	Enrichment p
BI.CD34_Primary_Cells_peaks.bed	H3K4me3	0.0107	0.3411	0.0774	1.04E-05	32.03	7.27	1.04E-05	5.93E-07	1.52E-07	3.898	9.70E-05
UCSD.Thymus_peaks.bed	H3K4me3	0.0094	0.3058	0.0689	8.97E-06	32.51	7.32	8.97E-06	5.81E-07	1.68E-07	3.457	5.46E-04
BI.CD34_Cultured_Cells_peaks.bed	H3K4me3	0.0109	0.3177	0.0751	2.31E-05	29.08	6.87	2.31E-05	5.35E-07	1.58E-07	3.381	7.22E-04
UW.CD14_Primary_Cells_peaks.bed	H3K4me3	0.0084	0.2839	0.0722	8.37E-05	33.91	8.62	8.37E-05	5.99E-07	1.85E-07	3.244	1.18E-03
UW.Fetal_Thymus_peaks.bed	H3K4me3	0.0093	0.2774	0.0689	5.64E-05	29.90	7.43	5.64E-05	5.10E-07	1.68E-07	3.043	2.34E-03
UCSF-UBC.Peripheral_Blood_Mononuclear_Primary_Cells_peaks.bed	H3K4me3	0.0097	0.2968	0.0764	1.02E-04	30.53	7.85	1.02E-04	5.34E-07	1.88E-07	2.839	4.53E-03
BI.Adult_Kidney_peaks.bed	H3K4me3	0.0174	0.3135	0.0736	2.06E-05	18.00	4.23	2.06E-05	2.86E-07	1.01E-07	2.829	4.66E-03
UCSF-UBC.Penis_Foreskin_Keratinocyte_Primary_Cells_peaks.bed	H3K4me3	0.0192	0.3272	0.0729	7.17E-06	17.05	3.80	7.17E-06	2.72E-07	9.70E-08	2.803	5.06E-03
BI.Adult_Kidney_peaks.bed	H3K9ac	0.0108	0.2740	0.0665	3.78E-05	25.47	6.18	3.78E-05	4.44E-07	1.61E-07	2.752	5.92E-03
UCSD.Psoas_Muscle_peaks.bed	H3K4me3	0.0099	0.2898	0.0721	5.83E-05	29.23	7.27	5.83E-05	4.60E-07	1.67E-07	2.748	5.99E-03
UW.Fetal_Intestine_Small_peaks.bed	H3K4me3	0.0112	0.2964	0.0738	5.88E-05	26.48	6.59	5.88E-05	4.29E-07	1.61E-07	2.670	7.59E-03
UCSF-UBC.Breast_Myoepithelial_Cells_peaks.bed	H3K4me3	0.0139	0.2839	0.0692	4.10E-05	20.43	4.98	4.10E-05	2.95E-07	1.14E-07	2.587	9.69E-03
UCSD.Right_Ventricle_peaks.bed	H3K4me3	0.0108	0.2781	0.0706	8.23E-05	25.65	6.51	8.23E-05	3.76E-07	1.49E-07	2.523	0.012
BI.Duodenum_Mucosa_peaks.bed	H3K9ac	0.0151	0.2608	0.0629	3.37E-05	17.26	4.16	3.37E-05	2.69E-07	1.07E-07	2.522	0.012
UCSD.Adrenal_Gland_peaks.bed	H3K4me3	0.0034	0.2030	0.0665	2.27E-03	59.63	19.53	2.27E-03	9.68E-07	3.93E-07	2.465	0.014
UCSD.Aorta_peaks.bed	H3K4me3	0.0085	0.2473	0.0678	2.67E-04	28.98	7.95	2.67E-04	4.18E-07	1.73E-07	2.422	0.015
UCSD.Right_Atrium_peaks.bed	H3K4me3	0.0111	0.2721	0.0714	1.38E-04	24.46	6.42	1.38E-04	3.48E-07	1.44E-07	2.416	0.016
kidney	H3K27ac	0.0251	0.2213	0.0610	2.88E-04	8.80	2.43	2.88E-04	1.27E-07	5.31E-08	2.388	0.017
UCSD.Spleen_peaks.bed	H3K4me3	0.0042	0.2043	0.0632	1.23E-03	48.96	15.15	1.23E-03	7.79E-07	3.31E-07	2.350	0.019
UCSD.Left_Ventricle_peaks.bed	H3K4me3	0.0088	0.2500	0.0691	2.95E-04	28.30	7.82	2.95E-04	4.05E-07	1.73E-07	2.345	0.019
UW.CD19_Primary_Cells_peaks.bed	H3K4me3	0.0089	0.2619	0.0792	9.38E-04	29.57	8.94	9.38E-04	4.99E-07	2.13E-07	2.343	0.019
UCSF-UBC.Fetal_Brain_peaks.bed	H3K4me3	0.0129	0.3595	0.1006	3.52E-04	27.91	7.81	3.52E-04	5.11E-07	2.18E-07	2.342	0.019
BI.Adult_Kidney_peaks.bed	H3K4me1	0.0085	0.1162	0.0370	1.69E-03	13.67	4.35	1.69E-03	2.33E-07	9.96E-08	2.338	0.019
BI.CD3_Primary_Cells_peaks.bed	H3K4me3	0.0142	0.2412	0.0597	5.40E-05	17.00	4.21	5.40E-05	2.22E-07	9.52E-08	2.332	0.020
UCSF-UBC.Brain_Germinal_Matrix_peaks.bed	H3K4me3	0.0116	0.3755	0.1102	6.56E-04	32.50	9.54	6.56E-04	6.26E-07	2.71E-07	2.305	0.021
UCSD.Pancreas_peaks.bed	H3K4me3	0.0083	0.2524	0.0688	2.44E-04	30.23	8.24	2.44E-04	4.36E-07	1.89E-07	2.303	0.021
BI.Colonic_Mucosa_peaks.bed	H3K4me3	0.0120	0.2710	0.0718	1.59E-04	22.58	5.98	1.59E-04	3.29E-07	1.45E-07	2.277	0.023
UW.Fetal_Muscle_Leg_peaks.bed	H3K4me3	0.0098	0.2499	0.0677	2.23E-04	25.44	6.89	2.23E-04	3.54E-07	1.59E-07	2.228	0.026
UW.Fetal_Intestine_Large_peaks.bed	H3K4me3	0.0110	0.2593	0.0672	1.14E-04	23.65	6.13	1.14E-04	3.30E-07	1.51E-07	2.189	0.029
UCSD.Small_Intestine_peaks.bed	H3K4me3	0.0049	0.2263	0.0752	2.61E-03	45.74	15.19	2.61E-03	7.45E-07	3.41E-07	2.188	0.029
UCSF-UBC.CD8_Naive_Primary_Cells_peaks.bed	H3K4me3	0.0062	0.1872	0.0548	6.29E-04	30.24	8.85	6.29E-04	4.09E-07	1.89E-07	2.170	0.030
UCSD.Gastric_peaks.bed	H3K4me3	0.0059	0.2283	0.0717	1.45E-03	38.61	12.12	1.45E-03	5.75E-07	2.67E-07	2.153	0.031
UCSF-UBC.Placenta_Amnion_peaks.bed	H3K4me3	0.0064	0.2126	0.0645	9.77E-04	33.33	10.11	9.77E-04	4.70E-07	2.20E-07	2.133	0.033
UCSD.Sigmoid_Colon_peaks.bed	H3K4me3	0.0056	0.2235	0.0742	2.59E-03	40.10	13.31	2.59E-03	6.29E-07	3.03E-07	2.078	0.038
UCSF-UBC.Placenta_Chorion_Smooth_peaks.bed	H3K4me3	0.0087	0.2479	0.0735	7.49E-04	28.61	8.49	7.49E-04	4.33E-07	2.08E-07	2.077	0.038
UCSD.Lung_peaks.bed	H3K4me3	0.0056	0.2057	0.0669	2.11E-03	36.56	11.89	2.11E-03	5.20E-07	2.52E-07	2.066	0.039
BI.CD19_Primary_Cells_peaks.bed	H3K4me3	0.0121	0.2729	0.0847	1.28E-03	22.51	6.99	1.28E-03	3.53E-07	1.74E-07	2.034	0.042
UCSD.Ovary_peaks.bed	H3K4me3	0.0096	0.2467	0.0682	2.99E-04	25.68	7.10	2.99E-04	3.39E-07	1.68E-07	2.025	0.043
UW.Fetal_Placenta_peaks.bed	H3K4me3	0.0083	0.2654	0.0849	1.78E-03	32.16	10.29	1.78E-03	5.28E-07	2.67E-07	1.974	0.048
Duodenum_mucosa	H3K27ac	0.0259	0.2258	0.0720	1.71E-03	8.73	2.78	1.71E-03	1.19E-07	6.09E-08	1.961	0.050
BI.Pancreatic_Islets_peaks.bed	H3K9ac	0.0062	0.2014	0.0658	2.19E-03	32.26	10.53	2.19E-03	4.62E-07	2.38E-07	1.939	0.052
UCSF-UBC.Breast_Fibroblast_Primary_Cells_peaks.bed	H3K4me3	0.0058	0.2110	0.0709	2.93E-03	36.39	12.23	2.93E-03	5.23E-07	2.70E-07	1.935	0.053

Table S6. Study level information for the contributing exome studies

Type	Study Name / acronym	Full Study name	N	Mean age collected (SD)	Mean age ANM (SD)	SNP array	Analysis program	Specific Menopause question
eChip - euro discovery	1958BC	1958 National Child Development Study (also known as the 1958 Birth Cohort Study)	530	50. 0 (0. 26)	46. 2 (2. 69)	Illumina HumanExome-12v1_A Beadchip	SkatMeta v1. 4. 3	Variables from the surveys at 44/45 years and at 50 years were used to define menopause status: Period or menstrual bleeding in last 12 months? Reason periods stopped? Age at the time of last period? Year of last period? Whether currently on HRT? Before starting, had menstrual periods stopped? Age/date at the time of your last period before HRT? Age/Date at time of removal of uterus, removal of uterus and both ovaries, removal of uterus and one ovary, removal of both ovaries, removal of one ovary?
eChip - euro discovery	ARIC	Atherosclerosis Risk in Communities HapMap analysis	3500	55. 6 (5. 4)	48. 89 (6. 84)	exomechip v1	skatMetaVersion 1. 4. 3	Have you reached menopause? Age when menopause began? Cause of menopause?
eChip - euro discovery	CHS	Cardiovascular Health Study	1099	72. 67 (5. 63)	49. 17 (4. 37)	Illumina ExomeChip v1. 0	seqMeta	How old were you at the time of your last natural period (menopause)?
eChip - euro discovery	Fenland		239	na	49. 6 (4. 1)	Illumina HumanCoreExome	skatMETA	How old were you when you stopped having your periods (i. e. your age at menopause)?
eChip - euro discovery	FHS	Framingham Heart Study	1707	collected prospectively	49. 9 (3. 5)	Illumina HumanExome BeadChip	skatMeta	Have your periods stopped for one year or more?Age periods stopped?;cause periods stopped?
eChip - euro discovery	INGI-VB	Val Borbera Isolated Population Project	497	67. 0 (10. 8)	50. 6 (3. 5)	ExomeChip v1. 2 zCall	seqMeta	Have you reached the menopause (no menstrual period in the last 12 months)? Was this spontaneously?
eChip - euro discovery	InterAct Cases	European Prospective Investigation into Cancer & Nutrition - InterAct	571	60. 2 (5. 7)	49. 5 (3. 9)	Illumina HumanCoreExome	skatMETA	"If you are not still menstruating, how old were you when you stopped having your periods?"
eChip - euro discovery	InterAct Subcohort	European Prospective Investigation into Cancer & Nutrition - InterAct	853	60. 0 (5. 9)	49. 5 (3. 7)	Illumina HumanCoreExome	skatMETA	"If you are not still menstruating, how old were you when you stopped having your periods?"
eChip - euro discovery	KORA	Cooperative Health Research in the Region of Augsburg (follow-up 4)	497	64. 49 (8. 68)	49. 77 (3. 87)	Illumina exomechip version 1. 0	skatMeta 1. 4. 3	Did you have a menstrual period in the past 12 months? How old were you when you had your last menstrual period? Did you ever take hormone replacement therapy? How old were you then you took hormone replacement therapy for the first time? How many months or years in total did you take hormone replacement therapy? Do you currently take hormone replacement therapy? Did you have a hysterectomy or bilateral oophorectomy?
eChip - euro discovery	MESA	Multi-Ethnic Study of Atherosclerosis	1031	64. 43 (9. 37)	49. 31 (4. 29)	Illumina Human Exome 1. 0	SkatMeta	Have you gone through menopause? At what age did you go through menopause?
eChip - euro discovery	Rotterdam	Rotterdam Study I	507	69. 65 (7. 29)	49. 11 (4. 22)	Illumina Exome Chip v	skatmeta	Did you have a menstrual period in the past 12 months? Age at last menstrual period? For what reasons did the periods stop?
eChip - euro discovery	SHIP	Study of Health in Pomerania	405	64. 02 (8. 54)	50. 14 (4. 04)	Illumina Human Exome 1. 0	PLINK, R (skatMeta)	How old have you been when your periods stopped? Have your periods stopped by natural menopause or due to operations or illness (natural/operation, illness/other)? Have you used hormones during your menopause or afterwards? (yes/no)
eChip - euro discovery	SHIP-TREND	Study of Health in Pomerania - TREND	485	62. 64 (8. 64)	50. 29 (4. 19)	Illumina Human Exome 1. 0	PLINK, R (skatMeta)	How old have you been when your periods stopped? Have both ovaries been removed (yes/no)? Have you been sterilized (yes/no)? Have you ever used hormone replacement therapy (yes/no)?
eChip - euro discovery	WGHS	Women's Genome Health Study	11664	54. 2 (7. 2)	50. 6 (3. 6)	Illumina EXOME v1. 1	SEQMETA	At what age did your natural periods cease?

Type	Study Name / acronym	Full Study name	N	Mean age collected (SD)	Mean age ANM (SD)	SNP array	Analysis program	Specific Menopause question
eChip - euro discovery	WHI	Women's Health Initiative	10123	66. 47 (NA)	50. 45 (NA)	exomechip v1	skatMetaVersion 1. 4. 3	Age of menopause: based on the derived variable MENO: Age at which participant went through menopause. Computed using Form 31, questions 5, 6. 1,10, 10. 1; Form 2, questions 7, 18, 18. 2; Form 43 (age first used HRT). Exclusions using the following questions: Did you ever have an operation to have one or both of your ovaries taken out? And How old were you when you had your last operation to remove an ovary? Did you ever have a hysterectomy? (This is an operation to take out your uterus or womb.) and How old were you when you had your hysterectomy?
eChip - euro discovery	Cambridge Cancer	The EMBRACE, SEARCH (breast cancer and ovarian cancer) and SIBS studies	1626	60. 7 (6. 28)	50. 5 (3. 79)	Illumina ExomeChip v1. 0	skatMeta	Have your periods stopped completely? If YES, how old were you when your periods stopped completely?. . . . . years old. What was the reason your periods stopped ? Natural menopause (change of life) / Hysterectomy (removal of womb) or both ovaries removed / X-ray treatment (radiotherapy) / Took medication that stopped periods / Other (please specify)
eChip - euro discovery	Amish	Old Order Amish Study	319	63. 1 (9. 1)	49. 3 (3. 8)	Illumina ainfinium Human Exome Chip	MMAP (http://edn. som. umaryland. edu/mmap/index. php) and SKAT	Have you reached menopause? Was your menopause natural or the result of surgery, radiation or chemotherapy? In what year or how old were you when you reached menopause?
eChip - euro discovery	EGCUT	Estonian Genome Center, University of Tartu	931	64. 07 (9. 27)	49. 86 (3. 85)	Illumina HumanExome-12v1-1	Plink; skatMeta; R	Have your periods stopped for 1 year or more? Age periods stopped? Cause periods stopped? Have you used hormonal medicaments due to menopause? When did you start using them?
eChip - euro discovery	Sardinia	SardiNIA	1258	60. 52 (11. 25)	49. 94 (4. 21)	Illumina HumanExome Chip	skatMeta	How old were you at the time of your last menstrual period (menopause)? Cause periods stopped? Hormon Replacement therapy?
eChip - euro discovery	Korcula	CROATIA_Korcula	302	62. 23 (NA)	49. 6(4. 11)	Illumina Human_Exome12v1_A	SeqMeta	How old were you when you had your last menstrual period?Are you having a regular menstrual cycle? Age at menopause? Have you had any type of surgery that could have induced menopause? Do you use HRT?
eChip - euro discovery	Generation Scotland	Generation Scotland:Scottish Family Health Study	882	60. 43 (8. 02)	49. 75 (3. 99)	Illumina OMNI_Express+ exomeSeq_Meta	SeqMeta	If you no longer have periods, what age were you when they stopped?
eChip - euro replication	deCODE		10157	birth year 1926. 9 (8. 8)	48. 3(4. 1)	Illumina HumanHap 300,370,610K; Omni-1 and Omni-Express	Logistic regression using allele count as a covariate and IMPUTE	How old were you when your menstruation ceased?

Table S7. The exome variants taken forward for replication with the results in both the discovery and replication cohorts.

		Discovery								Replication in deCODE (N=10,157)					Discovery + Replication							
Exome ID	rsID	MAF	Beta (minor allele)	SE	p-value	Gene	Chr	Pos (B37)	Variant Type	p-value	Beta	SE	Freq. %	Avg. Imp.	Avg. Freq.	Min. Freq.	Max Freq.	Effect	SE	p-value	Direction	Het. p-value
exm1019849	**rs75770066**	**0.0359**	**0.9103**	**0.0767**	**1.79E-32**	***HELB***	**12**	**66704225**	**non-synonymous**	**0.171**	**0.323**	**0.236**	**1.661**	**0.9711**	**0.034**	**0.0031**	**0.0542**	**0.8542**	**0.073**	**1.17E-31**	**+++-+++++++-+++-+++++**	**0.04999**
exm1019854	**rs148126992**	**0.0252**	**1.0343**	**0.0905**	**2.96E-30**	***HELB***	**12**	**66704274**	**non-synonymous**	**0.21585**	**2.163**	**1.748**	**0.061**	**0.45351**	**0.0252**	**0.0006**	**0.0364**	**1.0373**	**0.0904**	**1.69E-30**	**+++-++-++++++++?+++++**	**0.1157**
exm1556796	**rs140267842**	**0.0082**	**0.8044**	**0.1607**	**5.58E-07**	***SLCO4A1***	**20**	**61288593**	**non-synonymous**	**0.008605**	**0.729**	**0.277**	**1.155**	**0.99429**	**0.0091**	**0.0011**	**0.0138**	**0.7854**	**0.139**	**1.60E-08**	**++++++-++++-++++-++-+**	**0.2407**
exm621332	rs62445870	0.0291	0.3935	0.0897	1.14E-05	*FIGNL1*	7	50514904	non-synonymous	0.89998	0.024	0.191	2.286	0.98811	0.028	0.0167	0.1223	0.3268	0.0812	5.69E-05	++++++++++-++++--+++	0.6282
exm1057075	rs116907814	0.0015	-2	0.4738	2.44E-05	*SACS*	13	23929941	non-synonymous	-	-	-	-	-	-	-	-	-	-	-	-	-
exm728404	rs140584637	0.0004	-4.279	1.0351	3.57E-05	*NRBP2*	8	1.45E+08	non-synonymous	-	-	-	-	-	-	-	-	-	-	-	-	-
exm1023273	rs61754793	0.0096	0.8778	0.2126	3.65E-05	*NAV3*	12	78400847	non-synonymous	0.5923	0.319	0.596	0.225	0.97567	0.0088	0.0006	0.0322	0.8147	0.2003	4.73E-05	+++-+-+++-+++++?+???+	0.9503
exm235561	rs149252951	0.0208	-0.404	0.1006	6.00E-05	*MARCH7*	2	1.61E+08	non-synonymous	0.43388	-0.159	0.203	2.063	0.9783	0.0207	0.0028	0.034	-0.356	0.0902	8.04E-05	--+++-----+---+-++---	0.4213
exm1167443	rs148931336	0.019	0.3975	0.1049	0.0001507	*TLN2*	15	63017183	non-synonymous, ESP-nors	0.97104	-0.012	0.331	0.822	0.99711	0.018	0.0082	0.0423	0.3601	0.1	0.00032	++++-++++++++++++++-	0.2124
exm1149848	rs76010310	0.0171	0.3917	0.1109	0.0004118	*ZFYVE19*	15	41099819	non-synonymous	0.062379	-0.973	0.522	0.298	0.92934	0.0165	0.003	0.0297	0.3327	0.1085	0.00216	+++++-+-++++++++--++-	0.2189

Table S8. Conditional analysis of the GWAS and Exome chip signals in the WGHS cohort.

HELB **pairwise regression**						
single SNP p	0.84894	3.62E-05	0.281847	2.20E-05	< 2e-16	1.10E-15
	rs3741604	rs1183272	rs7397861	rs12371165	rs148126992	rs75770066
rs3741604	-	4.92e-10	0.289402	1.35e-05	< 2e-16	3.26e-16
rs1183272	3.21e-06	-	0.017345	0.001562	2.01e-15	4.4e-14
rs7397861	0.980191	3.43e-06	-	1.54e-05	< 2e-16	1.62e-15
rs12371165	0.323959	0.002617	0.175587	-	2.94e-14	2.3e-12
rs148126992	0.061347	0.008008	0.875366	0.16047	-	0.139523
rs75770066	0.111226	0.003488	0.550741	0.321942	0.001787	-

SLCO4A1 **pairwise regression**			
single snp p	0.060164	3.31E-05	0.000353
	rs13040088	rs2236553	rs140267842
rs13040088	-	2.78e-05	0.000401
rs2236553	0.049223	-	1.84e-05
rs140267842	0.069994	1.61e-06	-

column SNP significant

column SNP not significant

HELB Pairwise LD							
				D'			
		rs3741604	rs1183272	rs7397861	rs12371165	rs148126992	rs75770066
	rs3741604		0.771	0.238	0.497	0.992	1
	rs1183272	0.532		0.328	0.847	0.967	0.963
R^2	rs7397861	0.037	0.079		1	0.565	0.929
	rs12371165	0.035	0.092	0.277		0.658	0.941
	rs148126992	0.033	0.028	0.021	0.102		1
	rs75770066	0.025	0.02	0.041	0.151	0.728	

SLCO4A1 Pairwise LD				
		D'		
		rs2236553	rs13040088	rs140267842
	rs2236553		0.027	0.976
R^2	rs13040088	0		0.278
	rs140267842	0.029	0	

Table S9. Results from GRAIL analysis.

SNP	Candidate gene(s)	GRAIL p-value
rs4246511	*RHBDL2*	0.000787339
rs1635502	*EXO1*	8.29E-05
rs1411478	*MR1*	0.74475816
rs12146051	*RAD54L*	0.007480318
rs10183486	*TLK1*	0.032226033
rs704795	*SNX17*	0.52029297
rs7598194	*TLK1*	0.032226033
rs1800932	*MSH6*	7.25E-05
rs3771067	*TFPI*	0.78464513
rs16858210	*PARL*	0.002213848
rs4693089	*MRPS18C*	0.18058735
rs6856693	*ACSL1*	0.92897112
rs6836268	*ZFP42*	0.1848786
rs6861925	*UIMC1*	0.35749765
rs6897876	*SPRY4*	0.84378727
rs9790920	*CPEB4*	0.35654069
rs11738223	*SH3PXD2B*	0.67888672
rs427394	*POLS*	0.032563071
rs1046089	*MSH5*	0.001034726
rs9348724	*GCM2*	0.61970689
rs3134942	*MSH5*	0.00093333
rs3094222	*MDC1*	0.008762626
rs2720044	*STAR*	0.056467642
rs10957156	*CHD7*	0.26965429
rs1016674	*APTX*	0.039557852
rs11031006	*FSHB*	0.43432965
rs10734411	*CCDC73*	0.80582963
rs2277339	*PRIM1*	0.54295351
rs12371165	*GRIP1*	0.72164009
rs3736332	*PIWIL1*	0.07401536
rs551087	*UNQ1887*	0.091150299
rs1727326	*DENR*	0.76005839
rs4886238	*TDRD3*	0.42881542
rs1760940	*APEX1*	0.027823456
rs1054875	*FANCl*	0.17695237
rs9796	*INOC1*	0.44766549
rs10852344	*GSPT1*	0.35044628
rs9039	*C16orf72*	0.360595
rs2037075	*BRCA1*	0.04476199
rs1565923	*STARD3*	0.47476649
rs8070740	*RABEP1*	0.19012249
rs11668344	*BRSK1*	0.037490589
rs349306	*C19orf22*	0.53937836
rs10409520	*ZNF676*	0.99003236
rs16991615	*MCM8*	0.31596326
rs13040088	*C20orf59*	0.25970701
rs86796	*DMC1*	0.011959607
rs5762534	*CHEK2*	0.001872779
rs5762855	*CHEK2*	0.003742051

Table S10. The results from the default MAGENTA analysis.

(Note: This table shows top 40 most significant results. All results are available in published paper supplementary)

Data base	Gene set	Orig. Gene set size	EFF GS SIZE	# GENES ABS LIST	# GENES WO	# GENES REM	MED GENE SIZE KB	MEAN GENE SIZE	P-value 95% Cut-off	FDR 95% Cut-off	No. Exp. Genes above 95% cut-off	No. Obs. Genes above 95% cut-off	P-value 75% cut-off	FDR 75% cut-off	No. Exp. Genes above 75% cut-off	No. Obs. Genes above 75% cut-off	No. Genes flagged
PANTHER_BIOLOGICAL_PROCESS	**DNA_repair**	**169**	**134**	**28**	**6**	**1**	**37**	**66**	**9.90E-07**	**5.00E-04**	**7**	**24**	**9.38E-04**	**1.34E-01**	**34**	**50**	**0**
PANTHER_MOLECULAR_FUNCTION	**Exodeoxyribonuclease**	**15**	**12**	**1**	**2**	**0**	**53**	**69**	**2.00E-04**	**5.60E-03**	**1**	**5**	**1.33E-02**	**4.16E-01**	**3**	**7**	**0**
GOTERM	**G2/M transition DNA damage checkpoint**	**10**	**9**	**0**	**1**	**0**	**81**	**108**	**4.20E-05**	**6.40E-03**	**0**	**5**	**4.90E-02**	**4.78E-01**	**2**	**5**	**0**
PANTHER_BIOLOGICAL_PROCESS	**DNA_replication**	**155**	**94**	**54**	**7**	**0**	**30**	**54**	**7.40E-05**	**1.37E-02**	**5**	**15**	**9.97E-03**	**2.09E-01**	**24**	**34**	**0**
GOTERM	**DNA-dependent DNA replication**	**15**	**15**	**0**	**0**	**0**	**19**	**38**	**1.00E-04**	**1.68E-02**	**1**	**6**	**1.78E-02**	**4.62E-01**	**4**	**8**	**0**
GOTERM	**histone methylation**	**12**	**12**	**0**	**0**	**0**	**61**	**73**	**3.00E-04**	**2.39E-02**	**1**	**5**	**2.40E-03**	**3.85E-01**	**3**	**8**	**0**
PANTHER_MOLECULAR_FUNCTION	**Replication_origin_binding_protein**	**12**	**12**	**0**	**0**	**0**	**61**	**119**	**3.30E-03**	**4.00E-02**	**1**	**4**	**5.04E-02**	**4.59E-01**	**3**	**6**	**0**
PANTHER_MOLECULAR_FUNCTION	**DNA_helicase**	**76**	**59**	**14**	**3**	**0**	**52**	**77**	**7.00E-04**	**4.74E-02**	**3**	**10**	**1.18E-02**	**2.98E-01**	**15**	**23**	**0**
PANTHER_BIOLOGICAL_PROCESS	Protein_folding	186	130	50	4	2	17	29	1.10E-03	6.79E-02	7	16	8.32E-02	6.77E-01	33	40	0
PANTHER_BIOLOGICAL_PROCESS	Chromatin_packaging_and_remodeling	237	140	55	9	33	35	61	2.00E-04	7.22E-02	7	17	3.21E-02	4.27E-01	35	45	0
PANTHER_BIOLOGICAL_PROCESS	RNA_localization	33	32	0	1	0	62	77	3.50E-03	8.06E-02	2	6	7.00E-04	6.99E-02	8	17	0
PANTHER_BIOLOGICAL_PROCESS	Other_steroid_metabolism	13	11	2	0	0	18	18	1.35E-02	1.01E-01	1	3	2.90E-01	8.12E-01	3	4	0
PANTHER_BIOLOGICAL_PROCESS	Regulation_of_lipid,_fatty_acid_and_steroid_metabolism	27	26	0	1	0	26	43	7.90E-03	1.02E-01	1	5	4.13E-02	4.47E-01	7	11	0
PANTHER_BIOLOGICAL_PROCESS	DNA_recombination	44	36	4	3	1	37	72	9.00E-03	1.09E-01	2	6	2.22E-02	3.02E-01	9	15	0
PANTHER_MOLECULAR_FUNCTION	mRNA_polyadenylation_factor	16	16	0	0	0	46	64	6.70E-03	1.15E-01	1	4	2.76E-02	3.49E-01	4	8	0
GOTERM	double-strand break repair	36	35	0	1	0	42	72	2.00E-04	1.16E-01	2	8	1.64E-02	4.40E-01	9	15	0
REACTOME	FANCONI_ANEMIA_PATHWAY	17	16	0	1	0	52	53	1.00E-03	1.22E-01	1	5	1.87E-01	7.18E-01	4	6	0
Ingenuity	Role.of.BRCA1.in.DNA.Damage.Response	29	29	0	0	0	57	67	2.90E-03	1.39E-01	1	6	4.27E-02	5.54E-01	7	12	0
GOTERM	DNA binding	1480	1123	89	54	214	25	53	1.00E-04	1.57E-01	56	84	9.00E-04	3.29E-01	281	320	0
GOTERM	vesicle membrane	14	13	0	1	0	46	79	2.70E-03	1.58E-01	1	4	2.56E-02	4.50E-01	3	7	0
GOTERM	DNA repair	187	166	8	10	3	32	55	7.30E-05	1.64E-01	8	21	2.42E-02	4.84E-01	42	53	0
GOTERM	double-stranded DNA binding	69	67	0	2	0	24	48	5.00E-04	1.69E-01	3	11	4.02E-01	8.62E-01	17	18	0
PANTHER_BIOLOGICAL_PROCESS	Non-vertebrate_process	20	14	5	1	0	23	33	2.71E-02	1.76E-01	1	3	1.13E-01	7.30E-01	4	6	0
GOTERM	antigen processing and presentation of peptide antigen via MHC class I	11	4	7	0	0	15	21	1.25E-02	1.79E-01	0	2	2.69E-01	6.98E-01	1	2	0
GOTERM	DNA replication	146	139	0	4	3	31	55	1.00E-04	1.88E-01	7	18	1.12E-02	4.22E-01	35	47	0

Data base	Gene set	Orig. Gene set size	EFF GS SIZ E	# GENES ABS LIST	# GENES WO	# GENES REM	MED GENE SIZE KB	MEAN GENE SIZE	P-value 95% Cut-off	FDR 95% Cut-off	No. Exp. Genes above 95% cut-off	No. Obs. Genes above 95% cut-off	P-value 75% cut-off	FDR 75% cut-off	No. Exp. Genes above 75% cut-off	No. Obs. Genes above 75% cut-off	No. Genes flagged
GOTERM	3'-5' exonuclease activity	14	14	0	0	0	33	51	5.40E-03	1.88E-01	1	4	2.58E-01	7.78E-01	4	5	0
GOTERM	response to DNA damage stimulus	83	73	4	5	1	33	59	1.10E-03	1.92E-01	4	11	1.54E-02	4.53E-01	18	27	0
GOTERM	positive regulation of DNA repair	15	14	0	1	0	37	83	4.80E-03	2.00E-01	1	4	1.21E-01	6.37E-01	4	6	0
GOTERM	protein complex assembly	102	94	3	2	3	37	82	2.00E-04	2.04E-01	5	14	4.63E-02	5.65E-01	24	31	0
REACTOME	DNA_REPAIR	104	99	3	1	1	32	49	5.00E-04	2.08E-01	5	14	6.20E-03	3.61E-01	25	36	0
GOTERM	response to ionizing radiation	30	29	0	1	0	43	64	3.00E-03	2.08E-01	1	6	8.86E-02	5.99E-01	7	11	0
GOTERM	RNA helicase activity	10	9	1	0	0	44	70	8.60E-03	2.13E-01	0	3	4.91E-02	4.75E-01	2	5	0
GOTERM	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	16	16	0	0	0	27	42	6.90E-03	2.15E-01	1	4	1.88E-01	7.07E-01	4	6	0
GOTERM	endosome	203	184	4	10	5	35	57	9.00E-04	2.20E-01	9	20	9.74E-02	6.41E-01	46	54	0
GOTERM	DNA damage response, signal transduction resulting in induction of apoptosis	16	15	0	1	0	54	59	5.70E-03	2.24E-01	1	4	1.82E-02	4.70E-01	4	8	0
GOTERM	helicase activity	112	98	5	6	3	44	74	1.20E-03	2.26E-01	5	13	1.82E-02	4.61E-01	25	34	0
GOTERM	chromatin binding	139	125	5	8	1	31	62	1.70E-03	2.45E-01	6	15	4.42E-02	5.47E-01	31	40	0
GOTERM	ATP binding	1469	1250	43	58	118	48	90	3.00E-04	2.49E-01	63	87	3.60E-03	4.14E-01	313	348	0
GOTERM	RNA polymerase III transcription factor activity	10	10	0	0	0	30	41	1.27E-02	2.50E-01	1	3	7.71E-02	5.61E-01	3	5	0
GOTERM	male gonad development	36	32	1	3	0	22	35	4.40E-03	2.50E-01	2	6	1.56E-01	7.10E-01	8	11	0

Table S11. All eQTLs across all tissues with a significant association for the top SNPs.

Meno. Region	Meno. SNP	Best eQTL SNP	Meno. SNP eQTL p value	Best eQTL p value	Tissue	Gene	Effect	Allele	ArrayID
2	rs12142240	rs12137934	3.09E-11	8.09E-12	Skin (MuTHER)	*FAAH*			ILMN_1810915
2	rs12142240	rs12137934	2.58E-49	2.49E-50	Whole blood (CHARGE)	*FAAH*	14.76	C	1990471
2	rs12142240	rs12142240	2.54E-09	2.54E-09	CD14+ monocytes (24h LPS stimulated)	*FAAH*	6.16		1990471
2	rs12142240	rs12142240	1.30E-24	1.30E-24	Blood(Fehrmann et al)	*FAAH*			1990471
2	rs12142240	rs12142240	1.13E-08	1.13E-08	Bcells (CD19+)	*NSUN4*			ouS1E7kwoU6on7P6g8
2	rs12142240	rs12142240	1.27E-05	1.27E-05	Prefrontal cortex (Alzheimer's)	*RAD54L*			10023806317
2	rs12142240	rs12142240	5.80E-09	5.80E-09	Whole blood (Battle)	*RAD54L*			
2	rs12142240	rs12142240	1.67E-08	1.67E-08	Prefrontal cortex (all samples)	*RAD54L*			10023806317
3	rs1411478	rs12744212	9.05E-11	9.05E-11	Parietal lobe (ScanDB)	*STX6*			
3	rs1411478	rs1411478	3.33E-07	3.33E-07	Liver(Greenawalt)	*STX6*			10025902567
5	rs704795	rs1260345	3.18E-09	2.45E-09	Whole blood (CHARGE)	*EIF2B4*	5.92	A	5960546
5	rs704795	rs1563951	1.33E-07	6.87E-09	CD14+ monocytes (untreated)	*EIF2B4*	5.38		5960546
5	rs704795	rs1563951	7.48E-06	6.87E-09	CD14+ monocytes (untreated)	*EIF2B4*	4.54		6370494
5	rs704795	rs4665969	3.16E-05	1.67E-06	Skin (MuTHER)	*GTF3C2*			ILMN_1746457
5	rs704795	rs1060525	2.65E-19	2.65E-19	Bcells (CD19+)	*KRTCAP3*			ueSgoaH0qknIoDhInY
5	rs704795	rs11681351	1.86E-05	1.40E-06	Intestine (normal ileum)	*NRBP1*			
5	rs704795	rs1260320	3.63E-12	5.29E-13	CD14+ monocytes (untreated)	*NRBP1*	-7.19		430239
5	rs704795	rs11126999	5.68E-09	1.87E-09	CD14+ monocytes (2h LPS stimulated)	*NRBP1*	-6.07		430239
5	rs704795	rs12475426	8.14E-05	3.65E-05	Intestine (normal ileum)	*PPM1G*			
5	rs704795	rs2280737	6.50E-08	5.11E-08	CD14+ monocytes (untreated)	*PPM1G*	-5.52		3140008
5	rs704795	rs2280737	2.25E-06	4.30E-07	CD14+ monocytes (IFNg stimulated)	*PPM1G*	-4.82		3140008
5	rs704795	rs13472	7.07E-06	2.54E-06	CD14+ monocytes (24h LPS stimulated)	*PPM1G*	-4.58		3140008
5	rs704795	rs12475426	1.22E-15	1.42E-16	CD14+ monocytes (IFNg stimulated)	*SNX17*	-8.42		3360468
5	rs704795	rs12475426	1.24E-14	5.76E-16	CD14+ monocytes (24h LPS stimulated)	*SNX17*	-8.15		3360468
5	rs704795	rs2293571	2.29E-18	1.17E-18	CD14+ monocytes (2h LPS stimulated)	*SNX17*	-9.58		3360468
5	rs704795	rs704795	3.69E-05	3.69E-05	CD14+ monocytes (untreated)	*SNX17*	-4.18		3360468
6	rs1800932	rs3136284	1.34E-19	3.10E-20	Monocytes	*MSH6*			
7	rs930036	rs7606822	3.62E-04	1.52E-04	CD14+ monocytes (IFNg stimulated)	*GORASP2*	3.60		3390592
7	rs930036	rs10191270	5.02E-08	6.70E-10	NormalSkin	*TLK1*			210379_s_at
7	rs930036	rs10191270	6.49E-09	6.70E-10	NormalSkin	*TLK1*			211077_s_at
7	rs930036	rs1978592	9.00E-28	4.60E-38	LCL	*TLK1*			210379_s_at
7	rs930036	rs10210693	1.18E-08	2.58E-10	PsoriasisLesionalSkin	*TLK1*			211077_s_at
7	rs930036	rs10210693	6.92E-10	2.58E-10	PsoriasisLesionalSkin	*TLK1*			210379_s_at
7	rs930036	rs1529378	4.86E-07	3.65E-09	Liver (ScanDB)	*TLK1*			
9	rs4693089	rs11099599	3.42E-25	1.07E-28	Temporal cortex	*MRPS18C*			ILMN_2085903
9	rs4693089	rs11099599	1.24E-08	1.95E-11	Cerebellum	*MRPS18C*			ILMN_2085903
13	rs365132	rs365132	2.50E-09	2.50E-09	Prefrontal cortex (all samples)	*FGFR4*			10026394343
13	rs365132	rs365132	3.92E-08	3.92E-08	Prefrontal cortex (Alzheimer's)	*FGFR4*			10026394343
13	rs365132	rs402511	9.95E-28	5.22E-28	Lymph	*Hs.484258*			Hs.484258-S
13	rs365132	rs402511	5.40E-09	3.90E-09	Blood(Fehrmann et al)	*UIMC1*			4900079
13	rs365132	rs365132	2.91E-21	2.91E-21	Whole blood (Battle)	*UIMC1*			chr5:176332005-176433780(NM_1199297)
13	rs365132	rs2940521	1.20E-07	7.72E-12	Prefrontal cortex (all samples)	*ZNF346*			10025906891
13	rs365132	rs402511	3.29E-14	1.92E-14	Whole blood (CHARGE)	*ZNF346*	-7.59	G	5290376
13	rs365132	rs365132	1.51E-06	1.51E-06	Prefrontal cortex (Alzheimer's)	*ZNF346*			10023835095
13	rs365132	rs365132	1.30E-03	1.30E-03	Blood(Fehrmann et al)	*ZNF346*			Human_RefSeq-8_v2-3420373
13	rs365132	rs365132	1.41E-05	1.41E-05	Visual cortex (all samples)	*ZNF346*			10023835095
15	rs707938	rs707928	5.65E-05	3.11E-05	Intestine (normal ileum)	*C6orf26*			
15	rs707938	rs805305	9.31E-05	4.68E-07	Bcells (CD19+)	*DDAH2*			fuiuqN5UElKKR5IJ3c
15	rs707938	rs707928	7.45E-04	4.48E-04	Bcells (CD19+)	*IP6K3*			091_zoednUKkpInTg4
15	rs707938	rs707938	1.62E-04	1.62E-04	CD14+ monocytes (2h LPS stimulated)	*MSH5*	-3.84		6330440
15	rs707938	rs707938	1.94E-11	1.94E-11	Whole blood (Japanese, Narahara et al., n=298)	*PRRC2A*	-0.18		A_24_P641130
18	rs10957156	rs4237038	3.47E-20	2.09E-21	Whole blood (CHARGE)	*CHD7*	-9.20	G	1580487
19	rs4879656	rs13289835	9.52E-11	1.78E-11	Skin (MuTHER)	*APTX*			ILMN_1726752
19	rs4879656	rs13289835	1.45E-07	1.78E-11	Skin (MuTHER)	*APTX*			ILMN_2317348
19	rs4879656	rs2274767	3.47E-12	6.79E-15	Whole blood (CHARGE)	*APTX*	-6.96	A	1570138
19	rs4879656	rs1016674	1.04E-08	5.55E-09	Lymph	*APTX*			GI_28329426-A
19	rs4879656	rs10758180	3.16E-10	2.19E-14	LCL (MuTHER)	*SMU1*			ILMN_1764803
19	rs4879656	rs4879656	1.70E-04	1.70E-04	CD4+lymph	*SMU1*			
21	rs11031006	rs11031006	9.17E-05	9.17E-05	Liver(UChicago)	*C11orf46*			A_24_P73036
21	rs11031006	rs11031006	3.60E-03	3.60E-03	Liver(UWash)	*C11orf46*			5550307
24	rs3741604	rs3741604	2.01E-09	2.01E-09	Cerebellum (all samples)	*GRIP1*			10025927583
24	rs3741604	rs3741604	3.77E-19	3.77E-19	omental	*GRIP1*			10025927583

Meno. Region	Meno. SNP	Best eQTL SNP	Meno. SNP eQTL p value	Best eQTL p value	Tissue	Gene	Effect	Allele	ArrayID
24	rs3741604	rs3741604	2.99E-16	2.99E-16	SubCutAdipose(Greenawalt)	*GRIP1*			10025927583
24	rs3741604	rs3741604	1.31E-13	1.31E-13	Prefrontal cortex (Alzheimer's)	*GRIP1*			10025927583
24	rs3741604	rs3741604	1.39E-05	1.39E-05	Lung	*GRIP1*			100148797_TGI_at
24	rs3741604	rs3741604	9.99E-24	9.99E-24	Prefrontal cortex (all samples)	*GRIP1*			10025927583
24	rs3741604	rs3741604	2.27E-07	2.27E-07	Visual cortex (Huntington's)	*GRIP1*			10025927583
24	rs3741604	rs1009211	1.02E-08	8.65E-09	CD14+ monocytes (2h LPS stimulated)	*HELB*	-5.96		7320132
24	rs7397861	rs7487393	1.41E-07	1.26E-07	Whole blood (CHARGE)	*HELB*	-5.26	C	7320132
24	rs3741604	rs12146776	1.62E-34	4.01E-48	Monocyte-derived dendritic cells influenza virus treated 10h (Lee et al.)	*HELB*	0.61		
24	rs3741604	rs3741604	8.80E-06	8.80E-06	Prefrontal cortex (Huntington's)	*HELB*			10025909988
24	rs3741604	rs3741604	6.61E-32	6.61E-32	Prefrontal cortex (all samples)	*HELB*			10025909988
24	rs3741604	rs3741604	1.25E-09	1.25E-09	Parietal lobe (ScanDB)	*HELB*			
29	rs1713460	rs1713460	8.82E-04	8.82E-04	CD14+ monocytes (IFNg stimulated)	*NDRG2*	3.36		380228
29	rs1713460	rs1713460	1.37E-51	1.37E-51	CD14+ monocytes (untreated)	*NP*	-17.77		6840075
29	rs1713460	rs1713460	8.81E-22	8.81E-22	CD14+ monocytes (24h LPS stimulated)	*NP*	-10.44		6840075
29	rs1713460	rs1713460	5.11E-25	5.11E-25	CD14+ monocytes (IFNg stimulated)	*NP*	-11.25		6840075
29	rs1713460	rs1713460	6.64E-12	6.64E-12	Lymph	*NP*			GI_4557800-S
29	rs1713460	rs1713460	5.26E-21	5.26E-21	SubCutAdipose(Greenawalt)	*NP*			10023821825
29	rs1713460	rs1713460	6.85E-23	6.85E-23	Monocytes (CD14+)	*NP*			He6I5Uo.h2pfIH_TJI
29	rs1713460	rs1713460	1.10E-23	1.10E-23	Whole blood (Battle)	*PNP*			
30	rs9796	rs9796	3.64E-05	3.64E-05	Lung	*CHAC1*			100139505_TGI_at
30	rs9796	rs2899002	1.35E-28	1.16E-37	Whole blood (Wright, n=4,647)	*INO80*			11722331_a_at
30	rs9796	rs1942	1.57E-07	3.51E-10	LCL (MuTHER)	*ITPKA*			ILMN_1776516
32	rs9039	rs9039	4.25E-08	4.25E-08	omental	*AK057657*			10023850343
33	rs10852344	rs10852344	5.75E-05	5.75E-05	SubCutAdipose(Greenawalt)	*Contig760 _RC*			10023817744
33	rs10852344	rs11075030	6.51E-32	1.97E-33	CD14+ monocytes (2h LPS stimulated)	*GSPT1*	13.90		830259
33	rs10852344	rs11075030	9.21E-16	3.40E-16	CD14+ monocytes (IFNg stimulated)	*GSPT1*	8.46		830259
35	rs8070740	rs8072363	2.71E-10	5.45E-11	Cerebellum (ScanDB)	*RPAIN*			
35	rs8070740	rs1050390	1.49E-08	1.42E-09	Parietal lobe (ScanDB)	*RPAIN*			
36	rs2941505	rs2517955	1.67E-09	1.45E-09	Skin (MuTHER)	*PGAP3*			ILMN_1805636
36	rs2941505	rs11078919	1.07E-06	1.02E-06	Subc adipose (MuTHER)	*PGAP3*			ILMN_1805636
37	rs1799949	rs3765640	1.95E-72	1.97E-73	LCL (MuTHER)	*BRCA1*			ILMN_2311089
37	rs1799949	rs3765640	3.68E-66	1.97E-73	LCL (MuTHER)	*BRCA1*			ILMN_1738027
37	rs1799949	rs9912203	2.27E-27	8.82E-29	Skin (MuTHER)	*BRCA1*			ILMN_1738027
37	rs1799949	rs9912203	2.25E-23	8.82E-29	Skin (MuTHER)	*BRCA1*			ILMN_2311089
37	rs1799949	rs8176193	1.16E-15	3.41E-19	Subc adipose (MuTHER)	*BRCA1*			ILMN_1738027
37	rs1799949	rs8176322	8.31E-06	1.40E-06	Temporal cortex	*BRCA1*			ILMN_1738027
37	rs1799949	rs8176322	7.87E-06	1.40E-06	Temporal cortex	*BRCA1*			ILMN_2311089
37	rs1799949	rs8176193	6.33E-19	3.41E-19	Subc adipose (MuTHER)	*BRCA1*			ILMN_2311089
37	rs1799949	rs9911630	4.24E-09	2.26E-09	CD14+ monocytes (24h LPS stimulated)	*BRCA1*	6.07		2810465
37	rs1799949	rs16942	7.57E-07	1.65E-07	CD14+ monocytes (untreated)	*BRCA1*	5.03		2810465
37	rs1799949	rs8176297	1.19E-51	2.08E-66	Whole blood (CHARGE)	*BRCA1*	15.12	A	540411
37	rs1799949	rs8176297	3.56E-64	2.08E-66	Whole blood (CHARGE)	*BRCA1*	16.91	A	2810465
37	rs1799949	rs1799949	5.03E-08	5.03E-08	Lymph	*BRCA1*			GI_6552306-A
37	rs1799949	rs1799949	8.36E-04	8.36E-04	CD14+ monocytes (IFNg stimulated)	*BRCA1*	3.37		2810465
37	rs1799949	rs1060915	1.65E-11	1.51E-14	Intestine (normal ileum)	*NBR2*			
37	rs1799949	rs11653253	5.07E-08	4.80E-08	PsoriasisUninvolvedSkin	*NBR2*			1553992_s_at
37	rs1799949	rs1060915	1.05E-08	1.01E-08	NormalSkin	*NBR2*			1553992_s_at
37	rs1799949	rs7223460	9.63E-08	8.83E-09	Pancreatic islet cells (n=89)	*NBR2*	-5.83		
37	rs1799949	rs9912203	2.13E-16	3.98E-17	Subc adipose (MuTHER)	*RND2*			ILMN_1736533
37	rs1799949	rs11653253	4.38E-12	1.64E-12	Skin (MuTHER)	*RND2*			ILMN_1736533
37	rs1799949	rs2298861	1.69E-04	1.06E-05	CD14+ monocytes (2h LPS stimulated)	*VAT1*	-3.83		2600746
37	rs1799949	rs2298861	1.33E-06	5.67E-08	CD14+ monocytes (IFNg stimulated)	*VAT1*	-4.93		2600746
37	rs1799949	rs11650132	8.63E-12	4.16E-15	CD14+ monocytes (untreated)	*VAT1*	-7.05		2600746
37	rs1799949	rs9911630	2.59E-08	2.48E-09	Subc adipose (MuTHER)	*VAT1*			ILMN_1700690
37	rs1799949	rs2298862	1.11E-13	1.55E-14	Whole blood (CHARGE)	*VAT1*	-7.43	A	2600746
40	rs11668344	rs1172822	2.18E-06	3.55E-08	Subc adipose (MuTHER)	*BRSK1*			ILMN_2185845
42	rs13040088	rs13040088	7.05E-14	7.05E-14	Whole blood (Japanese, Narahara et al., n=298)	*DIDO1*	-0.12		A_23_P395426
42	rs13040088	chr20:615577 82:D	7.80E-06	6.57E-06	Pancreatic islet cells (n=89)	*DIDO1:01 8*	-4.76		
42	rs2236553	rs2236553	3.67E-05	3.67E-05	SchadtLiver	*HSS00253 359*			
44	rs763121	rs4560233	2.09E-11	1.45E-14	Monocytes	*CBY1*			
44	rs763121	rs2205802	6.06E-05	5.06E-06	Monocytes (CD14+)	*CBY1*			Kne_r7vqlJp6L093RU

Meno. Region	Meno. SNP	Best eQTL SNP	Meno. SNP eQTL p value	Best eQTL p value	Tissue	Gene	Effect	Allele	ArrayID
44	rs763121	rs6519120	1.30E-10	1.06E-15	CD14+ monocytes (untreated)	*CBY1*	-6.61		510100
44	rs763121	rs2072796	3.33E-08	1.26E-08	CD14+ monocytes (24h LPS stimulated)	*CBY1*	-5.68		510100
44	rs763121	rs5750630	7.91E-05	2.35E-06	Aortic endothelial cells (OxPAPC treated + unstimulated controls)	*CBY1*	0.08		
44	rs763121	rs12004	2.76E-09	2.76E-09	Whole blood (PaxGene) in Japanese	*DDX17*	0.62		A_24_P925611
44	rs763121	rs2267390	1.53E-17	2.01E-25	Whole blood (CHARGE)	*DDX17*	8.53	G	1780709
44	rs763121	rs1043402	1.37E-07	3.15E-08	CD14+ monocytes (2h LPS stimulated)	*DDX17*	5.45		1780709
44	rs763121	rs5845381	5.42E-73	1.88E-102	Whole blood (Wright, n=4,647)	*DDX17*			11715476_s_at
44	rs763121	rs763121			Liver(UChicago)	*DDX17*			A_24_P925611
44	rs763121	rs4821807	3.41E-11	1.08E-15	Whole blood (CHARGE)	*JOSD1*	-6.63	G	1500711
44	rs763121	rs6519120	2.80E-04	9.63E-05	CD14+ monocytes (24h LPS stimulated)	*JOSD1*	-3.68		1500711
44	rs763121	rs1980455	1.26E-07	2.27E-08	CD14+ monocytes (IFNg stimulated)	*JOSD1*	-5.40		1500711
44	rs763121	rs12166894	2.68E-04	2.13E-04	CD14+ monocytes (2h LPS stimulated)	*JOSD1*	-3.70		1500711
44	rs763121	rs2267390	1.11E-14	6.48E-22	Aortic endothelial cells (controls, unstimulated)	*KDELR3*	-0.40		
44	rs763121	rs2267390	1.46E-13	4.68E-20	Aortic endothelial cells (OxPAPC treated + unstimulated controls)	*KDELR3*	-0.39		
44	rs763121	rs138703	1.90E-31	2.40E-40	Monocytes	*UNC84B*			

Tissue	Count
CD14+ monocytes (24h LPS stimulated)	38
Whole blood	22
NormalSkin	11
Prefrontal cortex (all samples)	10
Subc adipose	9
Liver	6
LCL	5
Bcells (CD19+)	4
Intestine (normal ileum)	4
Lymph	4
Aortic endothelial cells	3
Cerebellum	3
Parietal lobe (ScanDB)	3
PsoriasisSkin	3
Temporal cortex	3
Lung	2
Pancreatic islet cells (n=89)	2
Visual cortex (all samples)	2
CD4+lymph	1
Monocyte-derived dendritic cells influenza virus treated 10h (Lee et al.)	1
Omental	1

Table S12. Details of the protein–protein connections identified from STRING.

Node 1	Node 2	Node 1 String ID	Node 2 String ID	Node1 external ID	Node 2 external ID	Neigh-borhood	Fusion	Co-occurence	Homo-logy	Co-expression	Experimental	Knowledge	Text-mining	Combined score
HLA-C	*KIR2DS4*	991362	986890	ENSP00000365402	ENSP00000340011	0	0	0	0	0	0.621	0.8	0.55	0.961
MIS12	*MLF1IP*	992362	980677	ENSP00000370557	ENSP00000281453	0	0	0	0	0	0	0.9	0.43	0.939
PTBP1	*POLR2E*	988213	976100	ENSP00000349428	ENSP00000215587	0	0	0	0	0.22	0	0.9	0.103	0.92
FYN	*GRB7*	989724	983571	ENSP00000357656	ENSP00000310771	0	0	0	0	0	0	0.9	0.347	0.93
HLA-B	*KIR3DL2*	996380	985162	ENSP00000402956	ENSP00000325525	0	0	0	0	0	0	0	0.917	0.917
CASC5	*MLF1IP*	986413	980677	ENSP00000335463	ENSP00000281453	0	0	0	0	0.518	0	0.9	0.245	0.958
POLR2H	*POLR2E*	981731	976100	ENSP00000296223	ENSP00000215587	0	0	0	0	0.332	0.999	0.9	0.513	0.999
BRCA1	*FANCI*	988359	983583	ENSP00000350283	ENSP00000310842	0	0	0	0	0.642	0	0.9	0.517	0.98
PSME3	*PSMD3*	981415	979427	ENSP00000293362	ENSP00000264639	0	0	0	0	0.157	0	0.9	0	0.91
EXO1	*RAD51*	983715	979855	ENSP00000311873	ENSP00000267868	0.104	0	0	0	0.853	0.237	0	0.613	0.952
EZH1	*CBX6*	996579	994337	ENSP00000404658	ENSP00000384490	0	0	0	0	0	0	0.9	0.138	0.908
STAT2	*OASL*	984098	978285	ENSP00000315768	ENSP00000257570	0	0	0	0	0.332	0	0.9	0.183	0.937
HK3	*PMM2*	981356	979895	ENSP00000292432	ENSP00000268261	0.074	0	0	0	0	0	0.9	0	0.901
NDUFB6	*NDUFS5*	992089	990673	ENSP00000369176	ENSP00000362058	0	0	0	0	0.376	0	0.9	0.426	0.959
LY6G6E	*C6orf25*	996983	996800	ENSP00000408299	ENSP00000406706	0	0	0	0	0	0	0.9	0.341	0.929
MKNK1	*EIF4G1*	990462	984207	ENSP00000361014	ENSP00000316879	0	0	0	0	0	0.846	0	0.66	0.944
LTB	*LTA*	995506	992687	ENSP00000395102	ENSP00000372793	0	0	0	0.751	0	0.937	0	0.946	0.952
HLA-B	*STAT2*	996380	984098	ENSP00000402956	ENSP00000315768	0	0	0	0	0	0	0.9	0.372	0.932
BRCA1	*TUBG1*	988359	977788	ENSP00000350283	ENSP00000251413	0	0	0	0	0.223	0.937	0.9	0.125	0.994
PFKFB3	*HK3*	992077	981356	ENSP00000369100	ENSP00000292432	0	0	0	0	0	0	0.9	0.244	0.919
POLR2H	*GTF3C2*	981731	979457	ENSP00000296223	ENSP00000264720	0	0	0	0	0.118	0	0.9	0.078	0.907
EIF4G1	*PSME3*	984207	981415	ENSP00000316879	ENSP00000293362	0	0	0	0	0.106	0	0.9	0.101	0.908
NDUFS5	*UQCRH*	990673	983414	ENSP00000362058	ENSP00000309565	0	0	0	0	0.816	0	0.9	0.236	0.984
B4GALT1	*GCNT2*	992071	979514	ENSP00000369055	ENSP00000265012	0	0	0	0	0	0	0.9	0.305	0.925
STK11	*BRSK1*	985097	983554	ENSP00000324856	ENSP00000310649	0	0	0	0.811	0	0	0.9	0.623	0.911
CASP3	*BIRC7*	983604	976263	ENSP00000311032	ENSP00000217169	0	0	0	0	0	0.845	0	0.852	0.975
USP7	*PPM1G*	987391	979453	ENSP00000343535	ENSP00000264714	0	0	0	0	0.076	0.641	0	0.81	0.928
SRSF9	*POLR2E*	976874	976100	ENSP00000229390	ENSP00000215587	0	0	0	0	0.126	0	0.9	0	0.906
U2AF2	*SRSF9*	983209	976874	ENSP00000307863	ENSP00000229390	0	0	0	0	0	0	0.9	0.505	0.947
RAD51	*MSH2*	979855	977012	ENSP00000267868	ENSP00000233146	0.131	0	0	0	0.436	0.62	0	0.613	0.912
BRCA1	*RAD51*	988359	979855	ENSP00000350283	ENSP00000267868	0	0	0	0	0.759	0.974	0.9	0.84	0.999
GRIP1	*PICK1*	993774	988219	ENSP00000381098	ENSP00000349465	0	0	0	0	0	0.621	0	0.954	0.981
FYN	*GP6*	989724	983320	ENSP00000357656	ENSP00000308782	0	0	0	0	0	0.937	0.9	0.9	0.999
MSH6	*MSH2*	977074	977012	ENSP00000234420	ENSP00000233146	0	0	0.525	0.737	0.828	0.999	0.72	0.987	0.999
CASC5	*OIP5*	986413	976412	ENSP00000335463	ENSP00000220514	0	0	0	0	0.67	0	0.9	0.18	0.969
NFX1	*U2AF2*	992031	983209	ENSP00000368856	ENSP00000307863	0	0	0	0	0	0	0.9	0.09	0.902
FYN	*PIK3R3*	989724	979026	ENSP00000357656	ENSP00000262741	0	0	0	0.544	0	0	0.9	0.129	0.903
NCR3	*EGFL8*	994979	994742	ENSP00000390131	ENSP00000388000	0	0	0	0	0	0	0.9	0.189	0.913
MICA	*HLA-DRB1*	997878	994065	ENSP00000416357	ENSP00000382599	0	0	0	0	0	0	0.9	0.46	0.942
TNF	*IL23A*	995272	976810	ENSP00000392858	ENSP00000228534	0	0	0	0	0	0	0	0.942	0.942
TCAP	*TNNT1*	983800	981319	ENSP00000312624	ENSP00000291901	0	0	0	0	0.112	0	0.9	0	0.905
FAF2	*DERL2*	978843	975851	ENSP00000261942	ENSP00000158771	0	0	0	0	0	0.62	0	0.794	0.916
IRF2	*STAT2*	993345	984098	ENSP00000377218	ENSP00000315768	0	0	0	0	0	0	0.9	0.689	0.966
BRCA1	*MSH2*	988359	977012	ENSP00000350283	ENSP00000233146	0	0	0	0	0.608	0.953	0	0.66	0.992
LY6G6F	*GRB7*	996897	983571	ENSP00000407535	ENSP00000310771	0	0	0	0	0	0.619	0	0.77	0.906
EXO1	*MSH2*	983715	977012	ENSP00000311873	ENSP00000233146	0	0	0	0	0.612	0.995	0	0.962	0.999
TNNI3	*TNNT1*	987148	981319	ENSP00000341838	ENSP00000291901	0	0	0	0	0.672	0.621	0.9	0.755	0.996
MIS12	*CASC5*	992362	986413	ENSP00000370557	ENSP00000335463	0	0	0	0	0	0.997	0.9	0.75	0.999
MDC1	*BRCA1*	992741	988359	ENSP00000373060	ENSP00000350283	0	0	0	0.397	0	0.931	0	0.751	0.961
VPS25	*CHMP5*	977998	976629	ENSP00000253794	ENSP00000223500	0	0	0	0	0	0	0.9	0.558	0.952

Node 1	Node 2	Node 1 String ID	Node 2 String ID	Node1 external ID	Node 2 external ID	Neigh-borhood	Fusion	Co-occurence	Homo-logy	Co-expression	Experimental	Knowledge	Text-mining	Combined score
KCNQ2	*KCNA4*	988621	985496	ENSP00000352035	ENSP00000328511	0	0	0	0.591	0	0	0.9	0.461	0.917
CASC5	*KNTC1*	986413	985458	ENSP00000335463	ENSP00000328236	0	0	0	0	0.669	0	0.9	0.203	0.97
RPL27	*RPL19*	977996	976669	ENSP00000253788	ENSP00000225430	0.162	0	0	0	0.906	0.35	0	0.22	0.952
GP1BA	*F12*	985602	977980	ENSP00000329380	ENSP00000253496	0	0	0	0	0	0.621	0.9	0.133	0.962
DDX17	*U2AF2*	993621	983209	ENSP00000380033	ENSP00000307863	0	0	0	0	0	0.974	0	0.201	0.977
MED16	*MED1*	985171	982309	ENSP00000325612	ENSP00000300651	0	0	0	0	0	0.915	0.9	0.264	0.992
PSMD2	*PSME3*	983484	981415	ENSP00000310129	ENSP00000293362	0	0	0	0	0.228	0	0.9	0.13	0.923
MDC1	*RAD51*	992741	979855	ENSP00000373060	ENSP00000267868	0	0	0	0	0	0.621	0	0.926	0.97
HLA-B	*TNF*	996380	995272	ENSP00000402956	ENSP00000392858	0	0	0	0	0	0	0	0.927	0.927
RAD54L	*RAD51*	990469	979855	ENSP00000361043	ENSP00000267868	0	0	0	0	0.81	0.621	0	0.605	0.967
BRE	*SHMT2*	987370	986152	ENSP00000343412	ENSP00000333667	0	0	0	0	0	0.926	0	0	0.925
EIF4EBP1	*EIF4G1*	986979	984207	ENSP00000340691	ENSP00000316879	0	0	0	0	0	0	0.9	0.965	0.996
TNNI3	*TCAP*	987148	983800	ENSP00000341838	ENSP00000312624	0	0	0	0	0.156	0	0.9	0.283	0.931
MED1	*THRA*	982309	979425	ENSP00000300651	ENSP00000264637	0	0	0	0	0	0.995	0	0.268	0.996
EIF4G1	*RPS15*	984207	977028	ENSP00000316879	ENSP00000233609	0	0	0	0	0	0	0.9	0.145	0.908
RAB2A	*GORASP2*	979013	977059	ENSP00000262646	ENSP00000234160	0	0	0	0	0.102	0.62	0.9	0.797	0.991
MED24	*MED10*	988080	978151	ENSP00000348610	ENSP00000255764	0	0	0	0	0	0.545	0.9	0.246	0.96
MIS12	*RCN1*	992362	975804	ENSP00000370557	ENSP00000054950	0	0	0	0	0	0.987	0	0	0.986
IRF2	*IFI35*	993345	977559	ENSP00000377218	ENSP00000246911	0	0	0	0	0	0	0.9	0.22	0.916
HTR3E	*HTR3C*	986420	984847	ENSP00000335511	ENSP00000322617	0	0	0	0.963	0	0	0.9	0.893	0.903
ETV4	*ERBB2*	984762	980001	ENSP00000321835	ENSP00000269571	0	0	0	0	0	0	0	0.902	0.902
DYNC1I2	*CSNK1E*	993659	988768	ENSP00000380308	ENSP00000352929	0	0	0	0	0	0	0.9	0.108	0.904
MED24	*MED1*	988080	982309	ENSP00000348610	ENSP00000300651	0	0	0	0	0	0.922	0.9	0.675	0.997
NDUFB6	*UQCRH*	992089	983414	ENSP00000369176	ENSP00000309565	0	0	0	0	0.489	0	0.9	0	0.945
HLA-B	*HLA-DRB5*	996380	991106	ENSP00000402956	ENSP00000364114	0	0	0	0.721	0	0	0.9	0.563	0.914
HSPA1B	*BAG1*	995294	976637	ENSP00000393087	ENSP00000224112	0	0	0	0	0	0.846	0	0.399	0.901
UIMC1	*BRCA1*	991563	988359	ENSP00000366434	ENSP00000350283	0	0	0	0	0	0.999	0.9	0.612	0.999
NDUFS5	*NDUFS7*	990673	977032	ENSP00000362058	ENSP00000233627	0	0	0	0	0.352	0	0.9	0.374	0.953
HLA-B	*IRF2*	996380	993345	ENSP00000402956	ENSP00000377218	0	0	0	0	0	0	0.9	0.426	0.938
RPL27	*RPS15*	977996	977028	ENSP00000253788	ENSP00000233609	0.183	0	0	0	0.775	0.388	0.9	0.241	0.988
MED16	*MED10*	985171	978151	ENSP00000325612	ENSP00000255764	0	0	0	0	0	0.545	0.9	0.43	0.97
CHEK2	*MSH2*	992579	977012	ENSP00000372023	ENSP00000233146	0	0	0	0	0.358	0.846	0	0.341	0.926
KIR3DL1	*HLA-C*	993214	991362	ENSP00000375608	ENSP00000365402	0	0	0	0	0	0	0.8	0.927	0.984
MLF1IP	*OIP5*	980677	976412	ENSP00000281453	ENSP00000220514	0	0	0	0	0.798	0	0.9	0.318	0.984
ATPAF1	*ATP5B*	990458	978858	ENSP00000361005	ENSP00000262030	0	0	0	0	0	0.845	0	0.819	0.97
TNF	*IL2RA*	995272	992108	ENSP00000392858	ENSP00000369293	0	0	0	0	0	0	0	0.916	0.916
KNTC1	*TUBG1*	985458	977788	ENSP00000328236	ENSP00000251413	0	0	0	0	0.118	0	0.9	0	0.905
LSM2	*LSM1*	997604	983547	ENSP00000414006	ENSP00000310596	0	0	0	0	0.155	0.999	0	0.793	0.999
BRCA1	*BRE*	988359	987370	ENSP00000350283	ENSP00000343412	0	0	0	0	0	0.614	0.9	0.298	0.969
EIF2B4	*EIF2B5*	995485	980252	ENSP00000394869	ENSP00000273783	0	0	0	0	0.122	0.619	0.9	0.893	0.995
HLA-B	*KIR3DL1*	996380	993214	ENSP00000402956	ENSP00000375608	0	0	0	0	0	0	0	0.931	0.931
UIMC1	*BRE*	991563	987370	ENSP00000366434	ENSP00000343412	0	0	0	0	0	0.999	0.9	0.429	0.999
NDUFB6	*NDUFS7*	992089	977032	ENSP00000369176	ENSP00000233627	0	0	0	0	0.368	0	0.9	0.422	0.958
CDK19	*MED24*	989795	988080	ENSP00000357907	ENSP00000348610	0	0	0	0	0	0.266	0.9	0.078	0.922
FAM175A	*UIMC1*	992232	991563	ENSP00000369857	ENSP00000366434	0	0	0	0	0	0.999	0.9	0.562	0.999

Node 1	Node 2	Node 1 String ID	Node 2 String ID	Node1 external ID	Node 2 external ID	Neigh-borhood	Fusion	Co-occurence	Homo-logy	Co-expression	Experimental	Knowledge	Text-mining	Combined score
PTBP1	*U2AF2*	988213	983209	ENSP00000349428	ENSP00000307863	0	0	0	0	0.152	0	0.9	0.751	0.975
UQCRH	*NDUFS7*	983414	977032	ENSP00000309565	ENSP00000233627	0	0	0	0	0.432	0	0.9	0	0.939
HTR3D	*HTR3E*	996663	986420	ENSP00000405409	ENSP00000335511	0	0	0	0.942	0	0	0.9	0.893	0.905
FAM175A	*BRE*	992232	987370	ENSP00000369857	ENSP00000343412	0	0	0	0	0	0.999	0.9	0.429	0.999
DDX17	*MIS12*	993621	992362	ENSP00000380033	ENSP00000370557	0	0	0	0	0	0.987	0	0	0.986
HLA-B	*IFI35*	996380	977559	ENSP00000402956	ENSP00000246911	0	0	0	0	0	0	0.9	0.157	0.91
TCAP	*PNMT*	983800	980003	ENSP00000312624	ENSP00000269582	0	0	0	0	0.611	0	0	0.779	0.908
COL20A1	*COL9A3*	988588	987113	ENSP00000351767	ENSP00000341640	0	0	0	0.667	0	0	0.9	0.147	0.902
PTBP1	*SRSF9*	988213	976874	ENSP00000349428	ENSP00000229390	0	0	0	0	0.076	0	0.9	0.462	0.943
HSPA1A	*HSPBP1*	991252	978143	ENSP00000364802	ENSP00000255631	0	0	0	0	0.088	0.813	0	0.513	0.905
OASL	*IFI35*	978285	977559	ENSP00000257570	ENSP00000246911	0	0	0	0	0.621	0	0.9	0.271	0.968
NDUFB6	*NDUFAF1*	992089	978568	ENSP00000369176	ENSP00000260361	0	0	0	0	0.134	0.62	0.9	0.425	0.977
HK3	*KHK*	981356	978593	ENSP00000292432	ENSP00000260598	0	0	0	0	0	0	0.9	0.096	0.903
RPL28	*RPL27*	996209	977996	ENSP00000401450	ENSP00000253788	0	0	0	0	0.733	0	0.9	0.264	0.977
NDUFAF1	*NDUFS7*	978568	977032	ENSP00000260361	ENSP00000233627	0	0	0	0	0.112	0.62	0.9	0.467	0.978
EIF4G1	*PSMD3*	984207	979427	ENSP00000316879	ENSP00000264639	0	0	0	0	0.203	0	0.9	0.075	0.916
ENSG00000236625	*RDBP*	996422	994813	ENSP00000403377	ENSP00000388779	0	0	0	0	0	0	0.9	0.078	0.901
OR4K13	*OR4K14*	984470	982857	ENSP00000319322	ENSP00000305011	0	0	0	0.957	0	0	0.9	0.75	0.903
STK11	*ETV4*	985097	984762	ENSP00000324856	ENSP00000321835	0	0	0	0	0	0	0.9	0.824	0.981
F12	*C1QBP*	977980	976689	ENSP00000253496	ENSP00000225698	0	0	0	0	0	0	0.9	0.514	0.948
HTR3D	*HTR3C*	996663	984847	ENSP00000405409	ENSP00000322617	0	0	0	0.926	0	0	0.9	0.893	0.906
PSMD2	*PSMD3*	983484	979427	ENSP00000310129	ENSP00000264639	0	0	0	0	0.579	0.796	0.9	0.248	0.992
TAC3	*KISS1R*	982243	977070	ENSP00000300108	ENSP00000234371	0	0	0	0	0	0	0.9	0.541	0.951
FAM175A	*BRCA1*	992232	988359	ENSP00000369857	ENSP00000350283	0	0	0	0	0	0.995	0.9	0.421	0.999
RPS15	*RPL19*	977028	976669	ENSP00000233609	ENSP00000225430	0.41	0	0	0	0.814	0.388	0	0.235	0.937
GSDMB	*ORMDL3*	983794	982841	ENSP00000312584	ENSP00000304858	0	0	0	0	0.656	0	0	0.751	0.908
CHEK2	*BRCA1*	992579	988359	ENSP00000372023	ENSP00000350283	0	0	0	0	0.315	0.974	0.9	0.782	0.999
MED24	*MED16*	988080	985171	ENSP00000348610	ENSP00000325612	0	0	0	0	0	0	0.9	0.419	0.937
HLA-B	*OASL*	996380	978285	ENSP00000402956	ENSP00000257570	0	0	0	0	0.151	0	0.9	0.083	0.911
CDK19	*MED10*	989795	978151	ENSP00000357907	ENSP00000255764	0	0	0	0	0	0.266	0.9	0.147	0.928
BRCA1	*MSH6*	988359	977074	ENSP00000350283	ENSP00000234420	0	0	0	0	0.366	0.885	0	0.584	0.965
NDUFS5	*NDUFAF1*	990673	978568	ENSP00000362058	ENSP00000260361	0	0	0	0	0.07	0.62	0.9	0.22	0.966
GRB7	*ERBB2*	983571	980001	ENSP00000310771	ENSP00000269571	0	0	0	0	0.756	0.977	0	0.916	0.999
EIF4G1	*PSMD2*	984207	983484	ENSP00000316879	ENSP00000310129	0	0	0	0	0.458	0	0.9	0.093	0.944
MDC1	*UIMC1*	992741	991563	ENSP00000373060	ENSP00000366434	0	0	0	0	0	0.846	0	0.614	0.936
GAD1	*ABAT*	988454	979894	ENSP00000350928	ENSP00000268251	0.137	0.001	0	0	0.093	0.104	0.9	0.411	0.946
POLR2H	*SRSF9*	981731	976874	ENSP00000296223	ENSP00000229390	0	0	0	0	0.085	0	0.9	0	0.902
ATP5B	*ATP5D*	978858	976088	ENSP00000262030	ENSP00000215375	0.472	0	0	0	0.748	0.8	0.9	0.779	0.999
RPL28	*RPS15*	996209	977028	ENSP00000401450	ENSP00000233609	0	0	0	0	0.82	0	0.9	0.341	0.986
BSG	*SLC16A8*	986166	984748	ENSP00000333769	ENSP00000321735	0	0	0	0	0	0	0.9	0.625	0.96
THRA	*NR1D1*	979425	977546	ENSP00000264637	ENSP00000246672	0	0	0	0.697	0.255	0	0.9	0.562	0.933
EPN1	*AP2M1*	996740	981384	ENSP00000406209	ENSP00000292807	0	0	0	0	0	0.229	0.9	0.27	0.935
STAT2	*IFI35*	984098	977559	ENSP00000315768	ENSP00000246911	0	0	0	0	0.417	0	0.9	0.318	0.954
GLS2	*CAD*	983519	979447	ENSP00000310447	ENSP00000264705	0	0	0	0	0.066	0	0.9	0.18	0.912
CDK19	*MED16*	989795	985171	ENSP00000357907	ENSP00000325612	0	0	0	0	0	0.266	0.9	0.078	0.922

Node 1	Node 2	Node 1 String ID	Node 2 String ID	Node1 external ID	Node 2 external ID	Neigh-borhood	Fusion	Co-occurence	Homo-logy	Co-expression	Experimental	Knowledge	Text-mining	Combined score
EIF4G1	*CASC3*	984207	979428	ENSP00000316879	ENSP00000264645	0	0	0	0	0	0	0.9	0.201	0.914
KNTC1	*MLF1IP*	985458	980677	ENSP00000328236	ENSP00000281453	0	0	0	0	0.776	0	0.9	0	0.976
HNF1A	*G6PC*	978283	978001	ENSP00000257555	ENSP00000253801	0	0	0	0	0	0	0.9	0.385	0.934
RPL28	*EIF4G1*	996209	984207	ENSP00000401450	ENSP00000316879	0	0	0	0	0	0	0.9	0.139	0.908
APC2	*MAPRE3*	977027	977009	ENSP00000233607	ENSP00000233121	0	0	0	0	0	0.621	0.54	0.677	0.935
VPS37B	*VPS25*	979804	977998	ENSP00000267202	ENSP00000253794	0	0	0	0	0	0	0.9	0.274	0.922
HLA-C	*KIR3DL2*	991362	985162	ENSP00000365402	ENSP00000325525	0	0	0	0	0	0	0.8	0.642	0.923
GAD1	*GLS2*	988454	983519	ENSP00000350928	ENSP00000310447	0.135	0	0	0	0	0	0.9	0.289	0.929
HSD17B6	*SRD5A1*	984386	980282	ENSP00000318631	ENSP00000274192	0	0	0	0	0.12	0	0.9	0.46	0.945
HSPA1A	*BAG1*	991252	976637	ENSP00000364802	ENSP00000224112	0	0	0	0	0	0.937	0	0.438	0.962
MICA	*TCF19*	997878	996220	ENSP00000416357	ENSP00000401548	0	0	0	0	0	0	0.9	0.159	0.91
IRF2	*OASL*	993345	978285	ENSP00000377218	ENSP00000257570	0	0	0	0	0	0	0.9	0.121	0.906
U2AF2	*POLR2E*	983209	976100	ENSP00000307863	ENSP00000215587	0	0	0	0	0.082	0	0.9	0	0.902
BRCA1	*UBE2S*	988359	979411	ENSP00000350283	ENSP00000264552	0	0	0	0	0	0	0.9	0.372	0.932

Table S13. MAGENTA results from the three custom pathways (POI, ovarian function, monogenic puberty).

Database	Gene Set	Orig. Gene set size	EFF GS SIZE	# GENES ABS LIST	# GENES WO SCORE	# GENES REM PROXI	Median Gene Sizr	Mean Gene Size (kb)	P-value 95% Cut-off	FDR 95% Cut-off	No. Exp. Genes above	No. Obs. Genes above	P-value 75% Cut-off	FDR 75% Cut-off	No. Exp. Genes above	No. Obs. Genes above	No. Genes Flagged	Flagged Genes
CUSTOM	POI Mendelian genes	31	23	1	7	0	18	39	2.49E-02	2.43E-02	1	4	3.58E-02	3.62E-02	6	10	30	*FOXL2, CD44, DAZL, DIAPH2, FOXO1, FMR1, AFF2, FSHR, NR5A1, GDF9, INHA, INHBA, SERPINE1, POLG, RPL10, EIF2B4, EIF2B2, EIF2B5, BMP15, NOG, STAG3, PGRMC1, DMC1, SOHLH2, EIF4ENIF1, SALL4, POF1B, NOBOX, HFM1, FIGLA*
CUSTOM	Menopause candidate genes	130	113	3	12	2	24	67	2.11E-01	1.39E-01	6	8	5.89E-02	5.16E-02	28	36	127	*ALOX12, AMH, AMHR2, AIRE, AR, BAX, BCL2L1, BDNF, BICD1, BMP2, BMP4, BMP7, BMP8B, BMPR1A, BMPR1B, BMPR2, FOXL2, CD44, CDKN1A, CDKN1B, CYP1B1, DAZL, DIAPH2, DNMT1, EIF2B1, EPS8, ESR1, FANCA, FANCC, FANCG, FGF8, FOXE1, FOXO1, FOXO3, FMR1, AFF2, FSHB, FSHR, NR5A1, GALT, GDF9, GDNF, GHR, GJA1, GJA4, HDC, INHA, INHBA, INHBB, KIT, SMAD1, SMAD3, SMAD5, MGAT1, KITLG, MOS, MSH4, NFE2L2, NGF, NTF4, NTRK1, NTRK2, SERPINE1, PDPK1, PIN1, PMM2, POLG, PTEN, PTPRO, RAD51C, RALB, RPA2, RPL10, CXCL12, SLC11A1, TAF4B, TIAL1, TSC2, XPNPEP2, ZFX, CXCR4, CGGBP1, EIF2B4, EIF2B3, EIF2B2, EIF2B5, BMP15, NOG, REC8, FST, STAG3, PGRMC1, DMC1, STRAP, DNAJC8, DICER1, SPO11, DAZAP1, EIF2C2, BBS9, SMPDL3B, YBX2, WNT4, SOHLH2, FANCL, ZNF654, NXF5, EIF4ENIF1, LHX9, CXCR7, SALL4, CNOT6, EPB41L5, TMEM35, CPEB1, POF1B, RERG, NOBOX, HFM1, ADAMTS19, RSPO1, C3orf38, ZAR1, NANOS3, FIGLA, SOHLH1, LHX8*
CUSTOM	Puberty (monogenic)	21	19	0	2	0	14	56	1.37E-02	1.82E-02	1	4	7.96E-02	7.98E-02	5	8	0	*KISS1R, CHD7, FGFR1, TAC3, BRWD2, FGF8, PROK2, KISS1, HS6ST1, SOX10, GNRH1, SEMA3A, LEPR, MKRN3, LEP, GNRHR, PROKR2, TACR3, NELF, NR0B1, KAL1*

Table S14. Details of the genes inputted for the custom POF MAGENTA pathway.

Gene	Other names	Cyto. band	Chr.	Start	Stop	Article citation	Reasons for being candidate gene
BMP15		Xp11.22	X	50653784	50659606	Pasquale et al 2006 J. Clin. Endocrinol. Metab.	7 of 166 unrelated patients with idiopathic POF found to be heterozygous for *BMP15* gene variants - consistent with BMP15 playing a role in folliculogenesis.
DAZL	*Deleted in azoospermia-like*	3p24	3	16628303	16647006	Tung et al 2006 Reprod. Biol. & Endocrin.	Identified 4 putative missense mutations in infertile men and women.
DIAPH2		Xq22	X	95939662	96855596	Bione et al 1998 AJHG	Family with POF identified with mutated *DIA* alleles that affects oogenesis and sterility. May be involved in ovarian follicle formation.
DMC1	*DISRUPTED MEIOTIC cDNA 1, YEAST, HOMOLOG OF*	22q13.1	22	38914954	38966189	Pittman et al 1998, Mol Cell; Mandon-Pépin 2008 EurJEndo	Ovaries from adult KO females were very small and grossly malformed. No follicles detected in these ovaries. These results indicate that while germ cells are indeed formed in Dmc1-/- ovaries, defects in oogenesis are manifest around pachytene stage or earlier, and that there must be subsequent death of oocytes to result in complete depletion in ovary by adulthood.
EIF2B2		14q24.3	14	75469612	75476292	Fogli et al 2003 AJHG	Mutations identified in patients with POF and white-matter abnormalities.
EIF2B4		2p23.3	2	27587221	27592919	Fogli et al 2003 AJHG	Mutations identified in patients with POF and white-matter abnormalities.
EIF2B5		3q27.1	3	183852810	183863098	Fogli et al 2003 AJHG	Mutations identified in patients with POF and white-matter abnormalities.
eIF4ENIF1	*eukaryotic translation initiation factor 4E nuclear import factor 1*	22q12.2	22	3188587	31885874	Kasippillai et al JCEM 2013 DOI: http://dx.doi.org/10.1210/jc.2013-1102	Dominant mutation in 7 affected members of 1 family, not in unaffecteds or controls. Not in 38 sporadic cases
FIGLA	*Folliculogenesis specific basic helix-loop-helix*	2p13.3	2	71004442	71017775	Zhao et al 2008 AJHG, Pangas et al 2006 PNAS	FIGLA oocyte specific gene. Found 3 mutations in 4 females of 100 Chinese females with POF. Missense c.11c-A(p.A4E), deletion c.15-36 del (p.G6fsX66), deletion c.419-421 delACA (p.140delN).
FMR1	*FRAXA*	Xq27.3	X	146990949	147003676	Murray et al 1998, J Med Ge	~5% of idiopathis POF cases carry *FMR1* premutation
FMR2	*FRAXE*	Xq28	X	147582139	148082192	Murray et al 1998, J Med Genet	147 women with idiopathic POF - an excess of small alleles with fewer than 11 repeats, and one small deletion.
FOXL2	*Forkhead TF*	3q22.3	3	138663067	138665982	Crisponi et al 2001 Nat Gen	Mutations cause BPES, a syndromic form of POF. Only 2 variations with presumed pathogenic effect were found in 320 unrelated patients with isolated POF [Bodega et al., 2004; De Baere et al.,2001, 2002; Harris et al., 2002]. Mutations in the coding region of the FOXL2 gene are not a major genetic cause of idiopathic nonsyndromic POF.
FOXO1A	*Forkhead box O1A*	13q14.1	13	41129803	41240734	Watkins et al 2006 Fert.Steril.	1.1% FOXO1A mutations identified in 90 POF patients.
FSHR		2p21-p16	2	49189653	49381630	Ghadami et al 2010, Mol Hum Reprod	A homozygous missense mutation, C566T, in follicle stimulation hormone receptor (*FSHR*) gene has been linked to premature ovarian failure. Female mice carrying mutated *FSHR* gene, called follitropin receptor knockout (FORKO), display similar phenotype. Intra-ovarian injection of an adenovirus expressing human *FSHR* gene is able to restore FSH responsiveness and reinitiate ovarian folliculogenesis as well as resume estrogen production in female FORKO mice.
GDF9	*Growth differentiation factor 9*	5q31.1	5	132196878	132200477	Palmer et al 2006 J. Clin. Endocrinol. Metab.	*GDF9* required for fertility. Novel variants in *GDF9* found in mothers of DZ twins.
HFM1	ATP-dependent DNA helicase homolog	1p22.2	1	91726323	91870426	Wang et al. NEJM 2014 370(10) DOI: 10.1056/NEJMc1310150	Recessive mutation in one familiy plus 1/69 sporadic POFs from China, mouse model and not in controls, homolgous recombination protein
INHA	*INHIBIN, ALPHA*	2q35	2	220436954	220440427	Shelling et al 2000, Hum Reprod	43 women with POF screened. 769G/A transition found in 3 patients.
INHBA	*INHIBIN, BETA A*	7p14.1	7	41728603	41742706	Shelling et al 2000, Hum Reprod	43 women with POF screened. A 1032C/T transition found in 1 patient.

Gene	Other names	Cyto. band	Chr.	Start	Stop	Article citation	Reasons for being candidate gene
LHR	*LUTEINIZING HORMONE/CHORIOGONADOTROPIN RECEPTOR*	2p21	2	48913921	48982880	Latronico et al 1996, NEJM	We report two novel homozygous inactivating nonsense and missense mutations of the LH-receptor gene — Arg^{554} stop $codon^{554}$ (TGA) and Ser^{616} $Tyr^{616,}$ respectively — in three pseudohermaphrodite 46,XY siblings with Leydig-cell hypoplasia and a 46,XX sister with amenorrhea, and a boy with micropenis and primary hypogonadism.
MSH5	*MutS, E. COLI, HOMOLOG OF, 5*	chr6_mcf_	6	3087458	3110642	de Vries et al 1999, Genes Dev. Mandon-Pépin 2008 EurJEndo	In Msh5−/− females, ovaries appeared rudimentary and were devoid completely of follicles and oocytes. P29S mutation in 2 women with sporadic POF
NOBOX	*NOBOX oogenesis homeob*	7q35	7	144096041	144107320	Qin et al 2007 AJHG, Pangas et al 2006 PNAS	*NOBOX* oocyte specific homeobox gene involved in folliculogenesis. 96 white women with POF showed 7 SNPs (rs757388, rs11769847, rs11979528), 4 novel mutations (p.Arg355His, p.Arg360Gln) found in *NOBOX*.
NOG	*Noggin*	17q23.2	17	54671060	54672951	Kosaki et al 2004, Fert & Steril	Japanese POF patient diagnosed with POF found to have heterozygous mutation (E48K) in Noggin.
NR5A1	*NUCLEAR RECEPTOR SUBFAMILY 5, GROUP A, MEMBER 1*	9q33	9	127243515	127269699	Lourenço et al 2010, New Eng J Med	Sequenced *NR5A1* in four families with histories of both 46,XY disorders of sex development and 46,XX primary ovarian insufficiency and in 25 subjects with sporadic ovarian insufficiency. Members of each of the 4 families and 2 of the 25 subjects with isolated ovarian insufficiency carried mutations in *NR5A1* gene. In-frame deletions and frameshift and missense mutations were detected.NR5A1 mutations are associated with 46,XX primary ovarian insufficiency and 46,XY disorders of sex development.
PAI-1	*plasminogen activator inhib*	7q22.1	7	100781707	100782532	Jeon YJ et al Fertil Steril 2014	Genotyping of 5 *PAI-1* polymorphisms: rs 2227631, rs1799889, rs6092, rs2227694 and rs7242 in 137 POI cases and 227 controls- SNPs were associated with POI-mechanism unknown
PGRMC1	*Progesterone Receptor Membrane Component-1*	Xq22-q24	X	118370211	118378428	Mansouri et al 2008 Hum Mol Gen	X-autosome translocation [t(X;11)(q24;q13)] identified in mother & daughter with POF. *H165R* mutation in female with idiopathic POF. Reduced levels of PGMRC1 may cause POF through impaired activation of P450 and increased apoptosis of ovarian cells.
POF1B		Xq21	X	84532395	84634748	Lacombe et al 2006	Lebanese family with 5 sisters with POF, found to be homozygous mutation (R329Q) in exon 10. Mutation influences pathogenesis of POF by altering POF1B binding to non-muscle filaments.
POLG	*POLYMERASE, DNA, GAM*	15q25	15	89859537	89878026	Luoma et al 2004 The Lancet	mtDNA gene. Mutations in *POLG* found in 7 families, women with progressive external ophthalmoplegia had menopause before 35yrs.
QM	*RIBOSOMAL PROTEIN L1*	Xq28	X	153626571	153630680	Massad-Costa et al 2007 Maturitas	5 mutations (4 were non-synonymous) identified in a patient with POF in Brazil.
SALL4	*SAL-LIKE 4*	20q13.13-	20	50400585	50419048	Wang et al 2009, Mol Hum Reprod	Screened *SALL4* coding regions for mutations in 100 Han Chinese women with non-syndromic ovarian failure and discovered two novel non-synonymous variants in the SALL4 gene: c.541G>A (p.Val181Met) and c.2449A>G. (p.Thr817Ala).
SOHLH2	*Spermatogenesis-and ooge*	13q13.3	13	36742345	36788752	Qin et al Fertil Steril 2014	Novel variants in *SOHLH2* in women with POI of Chinese and Serbian origin c/w ethnically matched controls
STAG3	*stromal antigen 3*	7q22.1	7	99775347	99812010	Caburet et al, N Engl J Med 2014;370:943-9. DOI: 10.1056/NEJMbr1309635	1bp deletion in consanguieneous family, KO mouse model, gene involved in pairing and segregation of chromosomes during meiosis

Table S15. Details of the genes inputted for the custom early menopause MAGENTA pathway.

Gene	Other names	Cyto. band	Chr.	Start	Stop	Article citation	Reasons for being candidate gene
BMP15		Xp11.22	X	50653784	50659606	Pasquale et al 2006 J. Clin. Endocrinol. Metab.	7 of 166 unrelated patients with idiopathic POF found to be heterozygous for *BMP15* gene variants - consistent with *BMP15* playing a role in folliculogenesis.
DAZL	Deleted in azoospermia-like	3p24	3	16628303	16647006	Tung et al 2006 Reprod. Biol. & Endocrin.	Identified 4 putative missense mutations in infertile men and women.
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DMC1	DISRUPTED MEIOTIC cDNA 1, YEAST, HOMOLOG OF	22q13.1	22	38914954	38966189	Pittman et al 1998, Mol Cell; Mandon-Pépin 2008 EurJEndo	Ovaries from adult KO females were very small and grossly malformed. No follicles detected in these ovaries. These results indicate that while germ cells are indeed formed in Dmc1-/- ovaries, defects in oogenesis are manifest around pachytene stage or earlier, and that there must be subsequent death of oocytes to result in complete depletion in ovary by adulthood.
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EIF2B5		3q27.1	3	183852810	183863098	Fogli et al 2003 AJHG	Mutations identified in patients with POF and white-matter abnormalities.
eIF4ENIF1	Eukaryotic translation initiation factor 4E nuclear import factor 1	22q12.2	22	3188587	31885874	Kasippillai et al JCEM 2013 DOI: http://dx.doi.org/10.1210/jc.2013-1102	Dominant mutation in 7 affected members of 1 family, not in unaffecteds or controls. Not in 38 sporadic cases
FIGLA	Folliculogenesis specific basic helix-loop-helix	2p13.3	2	71004442	71017775	Zhao et al 2008 AJHG, Pangas et al 2006 PNAS	FIGLA oocyte specific gene. Found 3 mutations in 4 females of 100 Chinese females with POF. Missense c.11c-A(p.A4E), deletion c.15-36 del (p.G6fsX66), deletion c.419-421 delACA (p.140delN).
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FOXL2	*Forkhead TF*	3q22.3	3	138663067	138665982	Crisponi et al 2001 Nat Gen	Mutations cause BPES, a syndromic form of POF. Only 2 variations with presumed pathogenic effect were found in 320 unrelated patients with isolated POF [Bodega et al., 2004; De Baere et al.,2001, 2002; Harris et al., 2002]. Mutations in the coding region of the FOXL2 gene are not a major genetic cause of idiopathic nonsyndromic POF.
FOXO1A	*Forkhead box O1A*	13q14.1	13	41129803	41240734	Watkins et al 2006 Fert.Steril.	1.1% *FOXO1A* mutations identified in 90 POF patients.
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GDF9	*Growth differentiation factor 9*	5q31.1	5	132196878	132200477	Palmer et al 2006 J. Clin. Endocrinol. Metab.	*GDF9* required for fertility. Novel variants in *GDF9* found in mothers of DZ twins.
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INHA	*INHIBIN, ALPHA*	2q35	2	220436954	220440427	Shelling et al 2000, Hum Reprod	43 women with POF screened. 769G/A transition found in 3 patients.
INHBA	*INHIBIN, BETA A*	7p14.1	7	41728603	41742706	Shelling et al 2000, Hum Reprod	43 women with POF screened. A 1032C/T transition found in 1 patient.
LHR	LUTEINIZING HORMONE/CHORIOGONADOTROPIN RECEPTOR	2p21	2	48913921	48982880	Latronico et al 1996, NEJM	We report two novel homozygous inactivating nonsense and missense mutations of the LH-receptor gene — Arg^{554} stop $codon^{554}$ (TGA) and Ser^{616} $Tyr^{616,}$ respectively — in three pseudohermaphrodite 46,XY siblings with Leydig-cell hypoplasia and a 46,XX sister with amenorrhea, and a boy with micropenis and primary hypogonadism.
MSH5	*MutS*, E. COLI, HOMOLOG OF, 5	chr6_mcf_h	6	3087458	3110642	de Vries et al 1999, Genes Dev: Mandon-Pépin 2008 EurJEndo	In Msh5−/− females, ovaries appeared rudimentary and were devoid completely of follicles and oocytes. *P29S* mutation in 2 women with sporadic POF
NOBOX	*NOBOX oogenesis homeobox*	7q35	7	144096041	144107320	Qin et al 2007 AJHG, Pangas et al 2006 PNAS	*NOBOX* oocyte specific homeobox gene involved in folliculogenesis. 96 white women with POF showed 7 SNPs (rs757388, rs11769847, rs11979528), 4 novel mutations (p.Arg355His, p.Arg360Gln) found in *NOBOX*.
NOG	*Noggin*	17q23.2	17	54671060	54672951	Kosaki et al 2004, Fert & Steril	Japanese POF patient diagnosed with POF found to have heterozygous mutation (E48K) in Noggin.

Gene	Other names	Cyto. band	Chr.	Start	Stop	Article citation	Reasons for being candidate gene
NR5A1	NUCLEAR RECEPTOR SUBFAMILY 5, GROUP A, MEMBER 1	9q33	9	127243515	127269699	Lourenço et al 2010, New Eng J Med	Sequenced *NR5A1* in four families with histories of both 46,XY disorders of sex development and 46,XX primary ovarian insufficiency and in 25 subjects with sporadic ovarian insufficiency. Members of each of the 4 families and 2 of the 25 subjects with isolated ovarian insufficiency carried mutations in NR5A1 gene. In-frame deletions and frameshift and missense mutations were detected.*NR5A1* mutations are associated with 46,XX primary ovarian insufficiency and 46,XY disorders of sex development.
PAI-1	Plasminogen activator inhibitor-1	7q22.1	7	100781707	100782532	Jeon YJ et al Fertil Steril 2014	Genotyping of 5 *PAI-1* polymorphism:s rs2227631, rs1799889, rs6092, rs2227694 and rs7242 in 137 POI cases and 227 controls- SNPs were associated with POI- mechanism unknown
PGRMC1	Progesterone Receptor Membrane Component-1	Xq22-q24	X	118370211	118378428	Mansouri et al 2008 Hum Mol Gen	X-autosome translocation [t(X;11)(q24;q13)] identified in mother & daughter with POF. *H165R* mutation in female with idiopathic POF. Reduced levels of PGMRC1 may cause POF through impaired activation of P450 and increased apoptosis of ovarian cells.
POF1B		Xq21	X	84532395	84634748	Lacombe et al 2006	Lebanese family with 5 sisters with POF, found to be homozygous mutation (R329Q) in exon 10. Mutation influences pathogenesis of POF by altering POF1B binding to non-muscle filaments.
POLG	POLYMERASE, DNA, GAMMA	15q25	15	89859537	89878026	Luoma et al 2004 The Lancet	mtDNA gene. Mutations in *POLG* found in 7 families, women with progressive external ophthalmoplegia had menopause before 35yrs.
QM	RIBOSOMAL PROTEIN L10	Xq28	X	153626571	153630680	Massad-Costa et al 2007 Maturitas	5 mutations (4 were non-synonymous) identified in a patient with POF in Brazil.
SALL4	*SAL-LIKE 4*	20q13.13-q1	20	50400585	50419048	Wang et al 2009, Mol Hum Reprod	Screened SALL4 coding regions for mutations in 100 Han Chinese women with non-syndromic ovarian failure and discovered two novel non-synonymous variants in the SALL4 gene: c.541G>A (p.Val181Met) and c.2449A>G. (p.Thr817Ala).
SOHLH2	Spermatogenesis-and oogenesis specific basic helix-loop-helix protein 2	13q13.3	13	36742345	36788752	Qin et al Fertil Steril 2014	Novel variants in SOHLH2 in women with POI of Chinese and Serbian origin c/w ethnically matched controls
STAG3	Stromal antigen 3	7q22.1	7	99775347	99812010	Caburet et al, N Engl J Med 2014;370:943-9. DOI: 10.1056/NEJMbr1309635	1bp deletion in consanguieneous family, KO mouse model, gene involved in pairing and segregation of chromosomes during meiosis
OCT4	Octomer binding protein 4 or *POU5F1*	6p21.31	6	2514038	2520393	Rajkovic et al 2004 Science	Reduced expression in newborn ovaries of NOBOX knockout mice.
ADAMTS19	A DISINTEGRIN-LIKE AND METALLOPROTEINASE WITH THROMBOSPONDIN TYPE 1 MOTIF, 19	5q31	5	128796103	129074376	Knauff et al 2009, Hum Mol Genet	GWAS on POF identified ADAMTS19 as a candidate gene. rs246246 (allele frequency P = 6.0 x 10(-7)) mapped to an intron of ADAMTS19, a gene known to be up-regulated in the female mouse gonads during sexual differentiation.
AIRE	AUTOIMMUNE REGULATOR	21q22.3	21	45705763	45718110	Laml et al 2002, Human Reprod Update	Causes autoimmune polyendocrinopathy-candidiasi-ectodermal dystrophy (APECED), an autosomal recessive disorder. Hypogonadism present in 60% patients in a 72-patient Finnish study and half of females with ovarian atrophy failed pubertal development.
ALOX12	ARACHIDONATE 12-OXIDOREDUCTASE	17p13.1	17	6899384	6914054	Liu et al 2010, Menopause	Study investigated ALOX12 gene for possible association with age at natural menopuase (ANM). Two SNPs, rs9904779 and rs434473 (encodes a replacement of asparagine by serine in the protein), were significantly associated with ANM (P = 0.022 and 0.033, respectively). Minor alleles of both SNPs seem to promote about 1.3- to 1.5-year earlier menopause and confer a 1.6 to 1.8 times higher risk for early menopause. ALOX12 gene seems to be associated with the timing of natural menopause in white women.
AMH	ANTI-MULLERIAN HORMONE	19p13.3-p13	19	2249113	2252072	Durlinger et al 1999 Endocrinology	AMH is produced by the ovary, but only postnatally. Mouse ovaries of AMH null females and those of females heterozygous for the AMH null mutation will show a relatively early depletion of their stock of primordial follicles.
AMHR2	ANTI-MULLERIAN HORMONE TYPE II RECEPTOR	12q13	12	53817641	53825312	Kevenaar et al 2007, Hum Reprod	2 large population-based cohorts of Dutch post-menopausal women (n = 2381 and n = 248). Observed association of AMHR2 -482 A > G (rs2002555) polymorphism with natural age at menopause suggests role for AMH signaling in usage of primordial follicle pool in women.
AR	Androgen Receptor	Xq11.2-q12	X	66763874	66944119	Shiina et al 2006 PNAS	Female AR knockout mice developed POF with aberrant ovarian gene expression, have lower follicle numbers, impaired mammary development and reduced amount of pups per litter.

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AR	Androgen Receptor	Xq11.2-q12	X	66763874	66944119	Shiina et al 2006 PNAS	Female AR knockout mice developed POF with aberrant ovarian gene expression, have lower follicle numbers, impaired mammary development and reduced amount of pups per litter.
BAX	BCL2-ASSOCIATED X PROTEIN	19q13.3-q13	19	49458117	49464519	Greenfield et al 2007, Reproduction	In mice Bax deletion results in delayed vaginal opening and altered follicular growth. Young adult Bax-deficient ovaries contained increased numbers of primordial follicles and a trend towards reduced numbers of growing follicles
BCL2L1	Bcl-X	20q11.21	20	29,717,425	29,773,430		Primordial germ cell migration and proliferation
BDNF	BRAIN-DERIVED NEUROTROPHIC FACTOR	11p13	11	27528399	27699348	Paredes et al 2004, Dev Biol	BDNF expressed in the infantile mouse ovary. Show that the ovaries of these mice or those lacking both NT-4 and BDNF suffer a stage-selective deficiency in early follicular development that compromises the ability of follicles to grow beyond the primary stage.
BICD	BICAUDAL D, DROSOPHILA, HOMOLOG OF, 1	12p11.2-p11	12	32260185	32531140	Swan & Suter 1996, Development	Bicaudal-D (Bic-D) gene is required early in Drosophila oogenesis for the differentiation of an oocyte. Bic-D is required for the localization of specific mRNAs at both the anterior and posterior of the oocyte.
BMP2	BONE MORPHOGENETIC PROTEIN 2	20p12.3	20	6,696,745	6,708,910		Primordial germ cell migration and proliferation
BMP4	BONE MORPHOGENETIC PROTEIN 4	14q22-q23	14	54416457	54421270	Pierre et al 2004, J Molec Endocrin	Ovine study confirmed that treatment with BMP-4 decreased basal GC progesterone secretion and totally abolished FSH-stimulating action.
BMP7	BONE MORPHOGENETIC PROTEIN 7	20q13.1-q13	20	55743809	55841707	Lee et al 2004, Mol Reprod Dev	BMP-7 is one of the factors involved in primordial-primary follicle transition in the mouse ovary and it may play a role in expression of FSHR for further follicular development.
BMP8B	BONE MORPHOGENETIC PROTEIN 8B	1p34.2	1	39,996,490	40,027,120		Primordial germ cell migration and proliferation
BMPR1A	BONE MORPHOGENETIC PROTEIN RECEPTOR, TYPE IA	10q22.3	10	88516396	88684944	Silva et al 2005 Mol Reprod Dev	Gene expressed in primordial, primary & secondary follicles and in oocyte & granulosa cells of antral follicles in goat ovaries
BMPR1B	BONE MORPHOGENETIC PROTEIN RECEPTOR, TYPE IB	4q22-q24	4	95679128	96079592	Silva et al 2005 Mol Reprod Dev	Gene expressed in primordial, primary & secondary follicles and in oocyte & granulosa cells of antral follicles in goat ovaries
BMPR2	BONE MORPHOGENETIC PROTEIN RECEPTOR, TYPE II	2q33-q34	2	203241050	203432473	Silva et al 2005 Mol Reprod Dev	Gene expressed in primordial, primary & secondary follicles and in oocyte & granulosa cells of antral follicles in goat ovaries
C3orf38	*MGC26717(C3orf38)*	3p21	3	88,281,799	88,288,755	Rizzolio et al Hum genet (2007) 121:441	
CDKN1a	*p21*	6p21.2	6	36646459	36655108	Jirawatnotai et al 2003, JBC	Granulosa cells in ovary establish quiescence within several hours after ovulation-inducing LH surge, whereas they undergo differentiation into corpora lutea. Expression of Cdk inhibitors p21(Cip1/Waf1) and p27(Kip1) is up-regulated during this process, suggesting that these cell cycle inhibitors are involved in restricting proliferative capacity of differentiating granulosa cells. Cooperation of p27(Kip1) and p21(Cip1) critical for withdrawal of granulosa cells from cell cycle, in concert with luteal differentiation and possibly culture-induced senescence.
CGGBP1		3p21	3	88101094	88199035	Rizzolio et al Hum genet (2007) 121:441	
CNOT6		5q35	5	179921417	180005405	Rizzolio et al Hum genet (2007) 121:441	
Connexin 37	Gap junction protein-α4	1p35.1	1	35258599	35261346	Yin et al 2009, Zygote	These data determine temporal gene expression of Cx37 in oocytes from follicules at different stages and indicate that the gene expression level of Cx37 in oocytes could be evaluated as a criterion to the regulatory mechanism of Cx37 in an in vitro model.

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Connexin 43	GAP JUNCTION PROTEIN, ALPHA-1	6q21-q23.2	6	121756745	121770872	Juneja et al 1999, Biol Reprod	In KO mice both sexes at time of birth, gonads of homozygous mutants were unusually small. This appears to be caused, at least in part, by a deficiency of germ cells. Folliculogenesis can proceed to primary (unilaminar) follicle stage in absence of Cx43 but that subsequent development is impaired. In neonatal ovaries of normal mice, Cx43 could be detected in the somatic cells as early as Day 1, when primordial follicles begin to appear, supporting the conclusion that Cx 43 is required for the earliest stages of folliculogenesis.
CPEB1	Cytoplasmic polyadenylation element-binding protein 1	15q25.2	15	83211952	83316728	Welk et al 2001 Gene	CPEB RNA expressed in immature oocytes and in adult ovaries.
CXCL12	Stromal cell-derived factor 1	10q11.1	10	44871366	44880542	Ara et al 2003 PNAS	PGCs have cell-surface expression of CXCR4 and, in SDF-1(-/-) mice, PGCs undergo directed migration through tissues of embryos, but the numbers of PGCs in the gonads are significantly reduced. Show essential role for SDF-1 in murine PGC development likely by controlling colonization of the gonads by PGCs.
CXCR4	CHEMOKINE, CXC MOTIF, RECEPTOR 4	2q21	2	136871920	136875725	Molyneaux et al 2003 Development	Results show that the SDF1/CXCR4 interaction is specifically required for colonization of gonads by primordial germ cells, but not for earlier stages in germ cell migration.
CXCR7	CHEMOKINE, CXC MOTIF, RECEPTOR 7	2q37	2	237478380	237490992	Mahabaleshwar et al 2008 Cell Adh Migr	Primordial Germ Cell (PGC) migration in zebrafish is guided by SDF-1a. CXCR7 acts as a high-affinity decoy receptor and facilitates the migration of PGCs by shaping the distribution of the chemokine in the environment.
CYP1B1	Cytochrome P450, family 1, subfamily B, poly-peptide 1			38294116	38337044	Hefler Hum Reprod. 2005	
DAZAP1	DAZ-ASSOCIATED PROTEIN 1	19p13.3	19	1407584	1435680	Pan et al 2005 Fert & Steril	DAZAP1 expressed in rat and human ovaries, may be involved in cell cycle regulation, oocyte maturation and luteal cell differentiation.
Dicer		14q31	14	95552565	95608085	Lei et al 2010, Mol Cell Endocrinol	Dicer is ribonuclease III for synthesis of mature functional microRNAs (miRNAs). In mouse ovary, Dicer1 protein was expressed in both oocyte and granulosa cells of follicle.Dicer1 plays important roles in follicular cell development through the differential regulation of gene expression.
DNAJC8		ip35.3		28527068	28559536	Rizzolio et al Hum genet (2007) 121:441	
DNMT1	DNA Methyltransferase 1	19p13.3-p13	19	10244023	10305755	Rajkovic et al 2004 Science	Reduced expression in newborn ovaries of NOBOX knockout mice.
EIF2B1		12q24.3	12	124105571	124118247		
EIF2B3		1p34.1	1	45316450	45452282		
EIF2C2	Argonaute2	8q24	8	141541265	141645645	Pepper et al 2009 PLoS ONE	*Drosophila* model showing dFMR1 regulation by Ago2 is observed in germ line causing multiple oocyte in a single egg chamber mutant phenotype.
EPB41L5		2q14.2	2	120,493,131	120,578,289	Rizzolio et al Hum genet (2007) 121:441	
EPS8		ch12p12.3	12	15773076	15942510	D.Toniolo personal communication	
ESR1	ESTROGEN RECEPTOR 1	6q25.1	6	152128454	152424406	Bretherick et al 2008, Fert & Steril	55 POF patients, 107 control women from the general population, and 27 control women who had proven fertility after age 37. Repeat in a promoter of the estrogen receptor alpha(ESR1) gene, POF patients had fewer (<18) short repeat alleles than did controls (P=.004 vs. combined controls). Genotypes consisting of two short alleles were found in 36.4% of control women but only 5.5% of POF patients (P<.0001 vs. combined controls). The ESR1 repeat may confer risk for POF in simple dominant manner in which carriers of long repeat have a relative risk of 9.7 (95% CI = 2.6 - 35.6).

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FANCA	FANCONI ANEMIA COMPLEMENTATION GROUP A GENE	16q24.3	16	89803959	89883065	Cheng et al 2000, Hum Mol Genet	The *Fanca* $^{-/-}$ females showed a severe fertility defect; they stopped breeding between 10 and 21 weeks of age. Ovaries of Fanca–/– females have few or almost no follicles. Young female mice are able to reproduce, although they have smaller litters. However, these mice cease breeding at an early age. This may be comparable to premature menopause in FA patients.
FANCC	FANCONI ANEMIA, COMPLEMENTATION GROUP C	9q22.3	9	97861338	98079991	Whitney et al 1996, Blood	The ovaries and uterus of mutant mice were abnormal when compared to litter mate controls. Mutant ovaries were much smaller in size and were almost completely devoid of follicles. There was marked ovarian cortical hypoplasia and hyperplasia of the interstitial cells.
FANCG	FANCONI ANEMIA, COMPLEMENTATION GROUP G	9p13	9	35073835	35080013	Koomen et al 2002, Hum Mol Genet	The ovaries from 36-week-old *Fancg* $^{-/-}$ female mice appeared to contain many interstitial cells. Few developing and some degenerated follicles were present.
FGF8	Fibroblast growth factor 8	10q24	10	103529887	103535827	Rajkovic et al 2004 Science	Reduced expression in newborn ovaries of NOBOX knockout mice.
FMR1	Fragile X mental retardation 1		X	146993481	147032645	Mallolas 2001	
FOXE1	FORKHEAD BOX E1	9q22	9	100615537	100618986	Watkins et al 2006 Molec Hum Rep	FOXE1 polyalanine tract length vary in length in POF patients and may predispose to POF.
FOXO3A (FKHRL1)	Forkhead Box O3A	6q21	6	108926596	109047524	Castrillon et al 2003, Science	Foxo3a-/- female mice exhibit a distinctive ovarian phenotype of global follicular activation leading to oocyte death, early depletion of functional ovarian follicles, and secondary infertility.
FSHB	FOLLICLE-STIMULATING HORMONE, BETA POLYPEPTIDE	11p13	11	30252563	30256823	Kumar et al 1997, Nat Genet	Ovaries and uteri from fshbm1/fshbm1 mice were small and thin. Ovaries lacked corpora lutea and failed to demonstrate any normal follicles beyond the primary (pre-antral) follicle stage - they did not undergo normal oestrous cycles. FSH-deficient females are infertile due to a block in folliculogenesis prior to antral follicle formation.
FST	Follistatin	5q11.2	5	52776595	52781902	Kimura et al 2010, Endocrinology	These results indicate that the FST isoforms have different activities in vivo, that the FST288-only isoform is sufficient for development, and that loss of FST303 and FST315 isoforms results in fertility defects that resemble activin hyperactivity and premature ovarian failure.
GALT	Galactose-1-phosphate uridylytransferase	9p13	9	34646635	34650571	Forges et al 2006 Hum. Reprod. Update	GALT mutations can cause galactosaemia, hypergonadotrophic hypogonadism in females and galactose-induced ovarian toxicity.
GDNF	GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR	5p13.1-p12	5	37815754	37839782	Dole et al 2008, Reproduction	Rat ovaries treated with GDNF contained a significant increase in developing follicles. Observations indicate that GDNF promotes primordial follicle development and mediates autocrine and paracrine cell-cell interactions required during folliculogenesis.
GHR	GROWTH HORMONE RECEPTOR	5p13-p12	5	42424026	42721925	Slot et al 2006, Reproduction	GH influences female fertility. Using GHR/GHBP-KO mice shows GH may play role, either directly or indirectly, via for instance IGF-I, in recruitment of primordial follicles into the growing pool. Furthermore, GH seems to protect antral follicles, directly or indirectly from undergoing atresia.
HDC	Histidine decarboxylase gene		15	50534144	50558223	Zhang et al. Biochem Biophys Res Commun. 2006 October 6; 348(4): 1378–1382.	
INHBB	*INHIBIN, BETA B*	2q14.2	2	121103719	121109383	Shelling et al 2000, Hum Reprod	
KIT		4q12	4	55,218,852	55,301,638	Gougeon, 2010, Ann Endocrinol	Primordial germ cell migration and proliferation
KITLG	Kit ligand	12q21.32	12	87,424,936	87,450,411	Hutt et al, 2006, MolHumRepro	Primordial germ cell migration and proliferation
LHX8	LIM homeobox gene 8	1p31.1	1	75594119	75627216	Pangas et al 2006 PNAS	Mouse model shows LHX8 is germ cell specific and critical regulator of oogenesis.
LHX9	LIM HOMEOBOX GENE 9	1q31-q32	1	197886517	197899273	Mazaud et al 2002, Gene Express Patterns	In the female rat, Lhx9 is highly expressed in epithelial ovigerous cords of the fetal ovary. Its expression is down-regulated as epithelial cells differentiate into granulosa cells during the process of folliculogenesis occurring at birth.

Gene	Other names	Cyto. band	Chr.	Start	Stop	Article citation	Reasons for being candidate gene
LSH	HELICASE, LYMPHOID-SPECIFIC	10q23.3-q24	10	96305574	96361855	De La Fuente et al 2006, Nat Cell Biol	Lsh is essential for completion of meiosis and transcriptional repression of repetitive elements in female gonad. Oocytes from Lsh knockout mice exhibit demethylation of transposable elements and tandem repeats at pericentric heterochromatin, as well as incomplete chromosome synapsis associated with persistent RAD51 foci and γH2AX phosphorylation The severe oocyte loss observed and lack of ovarian follicle formation, together with patterns of Lsh nuclear compartmentalization in the germ line, demonstrate that Lsh has a critical and previously unidentified role in epigenetic gene silencing and maintenance of genomic stability during female meiosis.
MGAT1		5q35	5	180217541	180242541	Rizzolio et al Hum genet (2007) 121:441	
MOS	V-mos Moloney murine sarcoma viral oncogene homolog	8q11	8	57025502	57026541	Rajkovic et al 2004 Science	Reduced expression in newborn ovaries of NOBOX knockout mice.
MSH4	*MutS* , E. COLI, HOMOLOG OF, 4	1p31	1	76262630	76378923	Kneitz et al 2000, Genes Dev	Meiosis was severely disrupted in mice Msh4 mutant females. Analysis of chromosomes in germ cells from embryonic and neonatal ovaries indicated that meiosis I was disrupted in Msh4−/− females. Loss of the oocyte pool resulted in the disruption of ovarian development and the complete loss of ovarian structures in the adult Msh4−/− female.
MSY2	Y-box binding protein 2	17p11.2-p13	17	7191571	7197876	Yang et al 2005 PNAS	RNA-binding protein. Juvenile MSY2 knockout mice have reduced follicle number and progression in ovaries and adults have increased oocyte loss, anovulation and multiple oocyte & follicle defects.
NANOS3	Nanos homolog 3 (Drosophila)	19p13.12	19	13987950	13991570	Yingying et al 2007 Fert. Steril.	NANOS3 encodes an RNA-binding protein and has a conserved function in germ cell development. The findings suggest that mutations in NANOS3 exons are rare in both Chinese and Caucasian women with premature ovarian failure.
NGF	NERVE GROWTH FACTOR, BETA SUBUNIT	1p13.1	1	115828537	115880857	Dissen et al 2001, Endocrinolgy	Ovaries from homozygote NGF-null (-/-) mutant animals, analyzed after completion of ovarian histogenesis, exhibited markedly reduced population of primary and secondary follicles in presence of normal serum gonadotropin levels, and an increased number of oocytes that failed to be incorporated into a follicular structure. Suggests that the delay in follicular growth observed in NGF(-/-) mice may be related to loss of proliferative signal provided by NGF to nonneural endocrine component of the ovary.
NT4	NEUROTROPHIN 4	19q13.3	19	49564399	49567119	Paredes et al 2004, Dev Biol	NT4 expressed in the infantile mouse ovary. Show that the ovaries of these mice or those lacking both NT-4 and BDNF suffer a stage-selective deficiency in early follicular development that compromises the ability of follicles to grow beyond the primary stage.
NTRK1	NEUROTROPHIC TYROSINE KINASE, RECEPTOR, TYPE 1	1q21-q22	1	156830671	156851642	Kerr et al 2009, Reproduction	Mouse model show that primordial follicle formation is decreased in absence of nerve growth factor (NGF) or its receptor NTRK1, and in absence of NTRK2, the receptor for neurotrophin-4 (NTF4) and brain-derived neurotrophic factor (BDNF). Results indicate that both NTRK1 and NTRK2 are necessary for the timely assembly of primordial follicles and for sustaining early follicular development.
NTRK2	NEUROTROPHIC TYROSINE KINASE, RECEPTOR, TYPE 2	9q22.1	9	87283466	87638505	Kerr et al 2009, Reproduction	Mouse model show that primordial follicle formation is decreased in absence of nerve growth factor (NGF) or its receptor NTRK1, and in absence of NTRK2, the receptor for neurotrophin-4 (NTF4) and brain-derived neurotrophic factor (BDNF). Results indicate that both NTRK1 and NTRK2 are necessary for the timely assembly of primordial follicles and for sustaining early follicular development.
NXF5	NUCLEAR RNA EXPORT FACTOR 5	Xq22.1	X	101087085	101112549	Bertini et al 2010, Fert & Steril	To characterize the breakpoints of a t(X;15) found in a woman with POF. Chromosome and FISH analysis revealed 46,XX, t(X;15)(Xq22.1;p11). FISH showed that NXF5 (nuclear RNA export factor 5) gene was contained in the clone spanning the breakpoint on the X chromosome.
p27kip1	CDKN1B	12p13.1-p12	12	12870302	12875305	Rajareddy et al 2007 Molec. Endo.	Mouse model suggest that p27 is important in determining mammalian ovarian development. This study therefore provides insight into ovary-borne genetic aberrations that cause defects in folliculogenesis and infertility in humans.

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PDPK1	3-@PHOSPHOINOSITIDE-DEPENDENT PROTEIN KINASE 1	16p13.3	16	2587970	2653188	Reddy et al 2009 Hum Mol Genet	PDK1 signaling in oocytes preserves reproductive lifespan by maintaining survival of ovarian primordial follicles. In mice lacking PDK1-encoding gene Pdk1 in oocytes, the majority of primordial follicles are depleted around onset of sexual maturity, causing POF during early adulthood.
PIN1	PEPTIDYL-PROLYL CIS/TRANS ISOMERASE, NIMA-INTERACTING, 1	19p13	19	9945999	9960358	Atchison et al 2003, Development	Pin1, is involved in the regulation of mammalian PGC proliferation. We discovered that both the male and female *Pin1*$^{-/-}$ mice had profound fertility defects. Investigation of the reproductive organs revealed significantly fewer germ cells in the adult *Pin1*$^{-/-}$ testes and ovaries than in wild type or heterozygotes, which resulted from *Pin1*$^{-/-}$ males and females being born with severely reduced number of gonocytes and oocytes
PMM2	PHOSPHOMANNOMUTASE 2	16p13.3-p13	16	8891670	8943193	Laml et al 2002, Human Reprod Update	Causes carbohydrate-deficient glycoprotein syndrome type 1, an autosomal recessive disorder. One of the symptoms of CDG1 is hypogonadism.
POG	PHD FINGER PROTEIN 9	2p16.1	2	58386380	58468515	Agoulnik et al 2002, Hum Mol Genet	Pog is critical for normal PGC proliferation, starting between 9.5 and 10.25 dpc when germ cells begin to migrate to the developing genital ridge.
PTEN	Phosphate and tensin homolog	10q23.31	10	89623195	89728531	Reddy et al 2008 Science	Mice lacking PTEN in oocytes causes entire primordial follicle pool to become activated, leading to depletion in early adulthood and POF.
PTHB1	PARATHYROID HORMONE-RESPONSIVE B1 GENE	7p14	7	33169152	33645680	Kang et al 2008, Hum Reprod	A first stage LD-based genome-wide association study was performed using 24 pairs of patients with POF and matched controls and a high-throughput BeadChip assay with 109365 SNPs. Part of chr 7p14 that contains the PTHB1 gene showing the strongest association. A POF-susceptible haplotype of PTHB1 (ht1, 'GAAAG', P = 0.00034) and a POF-resistant haplotype (ht2, 'TGTGC') were also identified. The association between POF and two PTHB1 SNPs (rs3884597 and rs6944723). Polymorphism of the non-synonymous SNP rs11773504 (Ala to Thr) and the repeated association of ht1 with POF suggest that PTHB1 may contribute to POF pathogenesis.
PTPRO		ch12p12.3	12	15475451	15750335	D.Toniolo personal communication	
RAD51C		17q	17	56769963	56811690	Meindl et al 2010, Nat Genet	In index cases from 1 1,100 German families with gynecological malignancies, we identified six monoallelic pathogenic mutations in RAD51C that confer an increased risk for breast and ovarian cancer.
RALB		2q14.2	2	120997640	121052284	Rizzolio et al Hum genet (2007) 121:441	
REC8	*REC8* , S. POMBE, HOMOLOG OF	14q11.2-q12	14	24641234	24649462	Xu et al 2005, Devel Cell	Rec8−/− mice displayed both in utero and postnatal growth retardation compared to their wt and heterozygous littermates. Rec8−/− day 5 neonatal ovaries (and later days up to adult ovaries) were characterized by a complete absence of oocytes and ovarian follicles and a dense fibrovascular stoma. Additionally, the genital tract of Rec8−/− females was involuted, probably as an indirect consequence of ovarian hormonal failure, secondary to the lack of follicles.
RERG		ch12p12.3	12	15260718	15374304		
RFPL4	RET FINGER PROTEIN-LIKE 4A	19q13.4	19	56270507	56274539	Suzumori et al 2003 PNAS	RFPL4 targets cyclin B1 for proteasomal degradation, a key aspect of oocyte cell cycle control during meiosis.
RPA2	replication protein A2	1p35.3	1	28218035	28241257	Rizzolio et al Hum genet (2007) 121:441	
RSPO1	R-Spondin-1	1p34.3	1	38076951	38100491	Chassot et al, + Tomizuka et al, 2008, HMG	Generating a mouse model, Rspo1 was shown to be the earliest known gene controlling the female genetic developmental program.
SMAD1	MOTHERS AGAINST DECAPENTAPLEGIC, DROSOPHILA, HOMOLOG OF, 1	4q28	4	146402951	146480323	Tremblay et al 2001, Development	Smad1-deficient embryos display a marked reduction in the number of primordial germ cells (PGCs) at 8.5 dpc. Loss of Smad1 affects either the initial specification of the PGC precursor population in the proximal epiblast or survival and/or proliferation of the allocated PGC population.

Gene	Other names	Cyto. band	Chr.	Start	Stop	Article citation	Reasons for being candidate gene
SMAD3	MOTHERS AGAINST DECAPENTAPLEGIC, DROSOPHILA, HOMOLOG OF, 3	15q21-q22	15	67358195	67487532	Tomic et al 2002, Biol Reprod	The results indicate that Smad 3-/- mice have reduced fertility compared with wild type mice. Results show Smad 3 may regulate growth of primordial follicles to the antral stage and regulate the expression of Bax and Bcl-2, but not Bcl-x, Cdk-2, and PCNA.
SMAD5	MOTHERS AGAINST DECAPENTAPLEGIC, DROSOPHILA, HOMOLOG OF, 5	5q31	5	135468536	135518422	Chang & Matzuk 2001, Mech Dev	Mouse model. It appears that the dosage of the Smad5 gene affects the size of the PGC founder population rather than the proliferation or survival of the PGCs. No PGCs were found in about 20% of the Smad5 null embryos. Our studies indicate that Smad5 is involved not only in PGC generation but also in PGC localization.
SMPDL3B		1p35.3	1	28261504	28285668	Rizzolio et al Hum genet (2007) 121:441	
SOHLH1	Spermatogenesis-and oogenesis specific basic helix-loop-helix protein 1	9q34.3	9	138585257	138591374	Pangas et al 2006 PNAS	Mouse model shows SOHLH1 a spermatogenesis & oogenesis TF, preferentially expressed in oocytes and required for oogenesis. Its disruption perturbs follicular formation and down regulates Nobox & Figla.
SOHLH2	Spermatogenesis-and oogenesis specific basic helix-loop-helix protein 2	13q13.3	13	36742347	36788752	Suzumori et al 2007 Curr. Med. Chem.	A transcription factor that regulates oocyte gene expression.
SPO11	*SPO11*, S. CEREVISIAE, HOMOLOG OF	20q13.2-q13	20	55904831	55919048	Romanienko & Camerini-Otero 2000, Mol Cell	Reduction in the number of follicles in Spo11-/- Female Mice. Defects can be seen as early as 15 dpc in the fetal ovaries of -/- mice that are entering the early stages of meiosis I.
STRAP		ch12p12.3	12	16035288	16056403	D.Toniolo personal communication	
TAF4B	TAF4b RNA polymerase II, TATA box binding protein- associated factor	18q11.2	18	23806409	23971647	Suzumori et al 2007 Curr. Med. Chem.;	A transcription factor that regulates oocyte gene expression.
TIAR	TIA1 CYTOTOXIC GRANULE-ASSOCIATED RNA-BINDING PROTEIN-LIKE 1	10q	10	121332978	121356541	Beck et al 1998, PNAS	Mutant mice lacking TIAR, an RNA recognition motif/ribonucleoprotein-type RNA-binding protein highly expressed in PGCs, fail to develop spermatogonia or oogonia. This developmental defect is a consequence of reduced survival of PGCs that migrate to the genital ridge around embryonic day 11.5 (E11.5).
TMEM35	*Xp18 (FLJ14084)*	Xq22.1	X	100333836	100351353	Cachot et al 2003 Gene	Flounder ovary cDNA library with a rainbow trout p53 probe led to isolation of p53-unrelated cDNA encoding an unknown 161 amino acid protein - was named Xp18. Gene expression significantly higher in ovary. Gene encoding the human protein is located on chromosome Xq22.1, a genome region involved in numerous genetic diseases including POF.
TSC2	TUBEROUS SCLEROSIS 2	16p13	16	2097990	2138712	Adhikari et al 2009, Mol Hum Reprod	In mutant mice lacking the Tsc2 gene in oocytes, the pool of primordial follicles is activated prematurely due to elevated mTORC1 activity in oocytes. This results in depletion of follicles in early adulthood, causing POF.
Wnt4	WINGLESS-TYPE MMTV INTEGRATION SITE FAMILY, MEMBER 4	1p35	1	22443800	22469519	Jeays-Ward, 2003, Development	Role of WNT4 in gonad development is to pattern the sex-specific vasculature and to regulate steroidogenic cell recruitment.
XPNPEP2	X-prolylaminopeptidase 2 or *APP2*	Xq26.1	X	128872946	128903525	Prueitt et al 2002 Cytogenet Genome Res	Balanced translocations with breakpoints in a critical region of the X chromosome, Xq13->q26, are associated with premature ovarian failure (POF). One translocation disrupted an aminopeptidase gene, XPNPEP2.
ZAR1	Zygote arrest 1	4p11	4	48492309	48496420	Rajkovic et al 2004 Science	Reduced expression in newborn ovaries of NOBOX knockout mice.
ZFX	ZINC FINGER PROTEIN, X-LINKED	Xp22.2-p21.	X	24169808	24232627	Luoh et al 1997, Development	Mutant mice (both males and females) were smaller, less viable, and had fewer germ cells than wild-type mice, features also found in human females with an XO karyotype (Turner syndrome). Zfx mutant mouse may prove to be a useful model system for human ovarian failure since both share several characteristics: diminished numbers of oocytes, shortened reproductive lifespan, and an association with the X chromosome.
ZNF654		3p21	3	88188254	88193815	Rizzolio et al Hum genet (2007) 121:441	

Table S16. GRASP and NHGRI look up of GWAS associated traits.

Query SNP	r2	D'	Meno. SNP	Meno. region	chr	pos(hg19)	PMID	P-value	Phenotype	Phenotype Description	Phenotype Categories	Date of Pub.	Journal	Title	Initial Sample Description	Replication Sample Description	GWAS ancestry Description	Total Samples (discovery + replication)	Total Discovery Samples	Suggested Gene	Function
rs1411478	1	1	rs1411478	3	1	180962282	21685912	3.50E-11	Progressive supranuclear palsy	Progressive supranuclear palsy	Neuro;Movement-related	19/06/2011	Nat Genet	Identification of common variants influencing risk of the tauopathy progressive supranuclear palsy.	1114 mostly EA cases, 3287 controls	1051 EA cases, 3560 EA controls	European	9012	4401	*(STX6)*	Intron
rs780110	0.82	1	rs704795	5	2	27685388	22885924	3.82E-20	Fasting blood glucose	Fasting glucose and insulin, and response to glucose in plasma	Quantitative trait(s);Type 2 diabetes (T2D);Plasma;Blood-related	12/09/2014	Nat Genet	Large-scale association analyses identify new loci influencing glycemic traits and provide insight into the underlying biological pathways.	Up to 133,010 individuals	NR	European	133010	133010	*(IFT172)*	Intron
rs1260333	0.51	0.95	rs704795	5	2	27748624	20081858	4.41E-08	Fasting blood glucose	Glucose homeostasis traits (fasting glucose, fasting insulin, HOMA-B, HOMA-IR)	Quantitative trait(s);Type 2 diabetes (T2D);Blood-related	17/01/2010	Nat Genet	New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk.	Up to 46186 EA individuals	up to 76558 EA individuals	European	122744	46186		
rs4665978	0.79	1	rs704795	5	2	27648726	21423719	1.11E-86	IFT172 cis expression in liver	Nonalcoholic fatty liver disease	Hepatic	10/03/2011	PLoS Genet	Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits.	7176 EA individuals	592 EA cases, 1405 EA controls	European	9173	7176		
rs1647276	1	1	rs704795	5	2	27688601	21423719	1.28E-222	IFT172 cis expression in omental fat	Nonalcoholic fatty liver disease	Hepatic	10/03/2011	PLoS Genet	Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits.	7176 EA individuals	592 EA cases, 1405 EA controls	European	9173	7176	*(IFT172)*	\
rs10205219	0.9	1	rs704795	5	2	27568565	21423719	1.58E-176	IFT172 cis expression in subcutaneous fat	Nonalcoholic fatty liver disease	Hepatic	10/03/2011	PLoS Genet	Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits.	7176 EA individuals	592 EA cases, 1405 EA controls	European	9173	7176	*(GTF3C2)*	\
rs1260341	1	1	rs704795	5	2	27663215	22001757	7.20E-241	IFT172 gene expression in adipose tissue	Liver enzyme concentrations (alanine aminotransaminase, alkaline phosphatase, gamme-glutamyl transferase), in plasma	Quantitative trait(s);Hepatic;Plasma	16/10/2011	Nat Genet	Genome-wide association study identifies loci influencing concentrations of liver enzymes in plasma.	Up to 52350 EA individuals, up to 8739 Indian Asian individuals	NR	Mixed	61089	61089	*(NRBP1)*	Intron
rs6547626	0.97	1	rs704795	5	2	27646770	22001757	3.80E-88	IFT172 gene expression in liver	Liver enzyme concentrations (alanine aminotransaminase, alkaline phosphatase, gamme-glutamyl transferase), in plasma	Quantitative trait(s);Hepatic;Plasma	16/10/2011	Nat Genet	Genome-wide association study identifies loci influencing concentrations of liver enzymes in plasma.	Up to 52350 EA individuals, up to 8739 Indian Asian individuals	NR	Mixed	61089	61089		
rs1260341	1	1	rs704795	5	2	27663215	22001757	1.00E-256	IFT172 gene expression in peripheral blood leucocytes	Liver enzyme concentrations (alanine aminotransaminase, alkaline phosphatase, gamme-glutamyl transferase), in plasma	Quantitative trait(s);Hepatic;Plasma	16/10/2011	Nat Genet	Genome-wide association study identifies loci influencing concentrations of liver enzymes in plasma.	Up to 52350 EA individuals, up to 8739 Indian Asian individuals	NR	Mixed	61089	61089	*(NRBP1)*	Intron

Query SNP	r2	D'	Meno. SNP	Meno. region	chr	pos(hg19)	PMID	P-value	Phenotype	Phenotype Description	Phenotype Categories	Date of Pub.	Journal	Title	Initial Sample Description	Replication Sample Description	GWAS ancestry Description	Total Samples (discovery + replication)	Total Discovery Samples	Suggested Gene	Function
rs7586601	0.76	1	rs704795	5	2	27584666	22001757	2.40E-09	NRBP1 gene expression in peripheral blood leucocytes	Liver enzyme concentrations (alanine aminotransaminase, alkaline phosphatase, gamme-glutamyl transferase), in plasma	Quantitative trait(s);Hepatic;Plasma	16/10/2011	Nat Genet	Genome-wide association study identifies loci influencing concentrations of liver enzymes in plasma.	Up to 52350 EA individuals, up to 8739 Indian Asian individuals	NR	Mixed	61089	61089		
rs1260333	0.51	0.95	rs704795	5	2	27748624	18439548	2.69E-09	Plasma C-reactive protein (female)	C-reactive protein (CRP) levels, in plasma, in women	Quantitative trait(s);Blood-related;Inflammation;CVD risk factor (CVD RF);C-reactive protein (CRP);Gender;Female;Plasma	24/04/2008	Am J Hum Genet	Loci related to metabolic-syndrome pathways including LEPR,HNF1A, IL6R, and GCKR associate with plasma C-reactive protein: the Women's Genome Health Study.	6345 EA women	NR	European	6345	6345		
rs1260333	0.51	0.95	rs704795	5	2	27748624	23362303	3.19E-08	Plasma palmitoleic acid	Fatty acid levels, in plasma	Quantitative trait(s);Blood-related;Plasma	29/01/2013	Circ Cardiovasc Genet	Genome-wide association study identifies novel loci associated with concentrations of four plasma phospholipid fatty acids in the de novo lipogenesis pathway: results from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium.	8,961 European ancestry individuals	NR	European	8961	8961		
rs1260333	0.51	0.95	rs704795	5	2	27748624	20802025	1.57E-13	Plasma protein-C levels	Protein-C levels, in plasma	Quantitative trait(s);Blood-related;Plasma	27/08/2010	Blood	Genome-wide association study identifies novel loci for plasma levels of protein C: the ARIC study.	8048 EA individuals	1376 EA individuals	European	9424	8048		
rs1260333	0.51	0.95	rs704795	5	2	27748624	23263486	5.01E-33	Serum urate	Urate (in serum), gout	Quantitative trait(s);Blood-related;Inflammation;Arthritis;Serum	23/12/2012	Nat Genet	Genome-wide association analyses identify 18 new loci associated with serum urate concentrations.	2,115 European ancestry cases, 67,259 European ancestry controls	1,036 European ancestry cases	European	70410	69374		
rs1260333	0.51	0.95	rs704795	5	2	27748624	21768215	6.36E-12	Serum urate	Urate (in serum), gout	Quantitative trait(s);Blood-related;Inflammation;Arthritis;Serum	18/07/2011	Hum Mol Genet	Genome-wide association study for serum urate concentrations and gout among African Americans identifies genomic risk loci and a novel URAT1 loss-of-function allele.	8651 African American individuals	1996 African American individuals	African	10647	8651		
rs1260333	0.51	0.95	rs704795	5	2	27748624	20884846	4.30E-13	Serum urate	Urate (in serum), gout	Quantitative trait(s);Blood-related;Inflammation;Arthritis;Serum;Gender;Male;Female	30/09/2010	Circ Cardiovasc Genet	Multiple genetic loci influence serum urate levels and their relationship with gout and cardiovascular disease risk factors.	28283 EA individuals	22054 EA women	European	50337	28283		
rs1260333	0.51	0.95	rs704795	5	2	27748624	20686565	1.22E-17	Total cholesterol	Lipid level measurements	CVD risk factor (CVD RF);Lipids	05/08/2010	Nature	Biological, clinical and population relevance of 95 loci for blood lipids.	Up to 100184 EA individuals	9705 South Asians, 15046 East Asians, 8061 African Americans, 7063 Europeans	Mixed	140059	100184		
rs7586601	0.76	1	rs704795	5	2	27584666	20686565	3.86E-09	Total cholesterol	Lipid level measurements	CVD risk factor (CVD RF);Lipids	05/08/2010	Nature	Biological, clinical and population relevance of 95 loci for blood lipids.	Up to 100184 EA individuals	9705 South Asians, 15046 East Asians, 8061 African Americans, 7063 Europeans	Mixed	140059	100184		

Query SNP	r2	D'	Meno. SNP	Meno. region	chr	pos(hg19)	PMID	P-value	Phenotype	Phenotype Description	Phenotype Categories	Date of Pub.	Journal	Title	Initial Sample Description	Replication Sample Description	GWAS ancestry Description	Total Samples (discovery + replication)	Total Discovery Samples	Suggested Gene	Function
rs780110	0.82	1	rs704795	5	2	27685388	20686565	4.74E-09	Total cholesterol	Lipid level measurements	CVD risk factor (CVD RF);Lipids	05/08/2010	Nature	Biological, clinical and population relevance of 95 loci for blood lipids.	Up to 100184 EA individuals	9705 South Asians, 15046 East Asians, 8061 African Americans, 7063 Europeans	Mixed	140059	100184	*(IFT172)*	Intron
rs4665978	0.79	1	rs704795	5	2	27648726	20686565	4.86E-09	Total cholesterol	Lipid level measurements	CVD risk factor (CVD RF);Lipids	05/08/2010	Nature	Biological, clinical and population relevance of 95 loci for blood lipids.	Up to 100184 EA individuals	9705 South Asians, 15046 East Asians, 8061 African Americans, 7063 Europeans	Mixed	140059	100184		
rs3739095	0.76	1	rs704795	5	2	27556721	20686565	4.61E-08	Total cholesterol	Lipid level measurements	CVD risk factor (CVD RF);Lipids	05/08/2010	Nature	Biological, clinical and population relevance of 95 loci for blood lipids.	Up to 100184 EA individuals	9705 South Asians, 15046 East Asians, 8061 African Americans, 7063 Europeans	Mixed	140059	100184	*(GTF3C2)*	Intron
rs1260333	0.51	0.95	rs704795	5	2	27748624	20339536	1.39E-11	Total cholesterol	Response to statin treatment (simvastatin, pravastatin, atorvastatin), change in cholesterol levels	Drug response;Quantitative trait(s);CVD risk factor (CVD RF);Lipids	22/03/2010	PLoS One	Genome-wide association of lipid-lowering response to statins in combined study populations.	3928 EA individuals	NR	European	3928	3928		
rs780110	0.82	1	rs704795	5	2	27685388	20339536	4.27E-08	Total cholesterol	Response to statin treatment (simvastatin, pravastatin, atorvastatin), change in cholesterol levels	Drug response;Quantitative trait(s);CVD risk factor (CVD RF);Lipids	22/03/2010	PLoS One	Genome-wide association of lipid-lowering response to statins in combined study populations.	3928 EA individuals	NR	European	3928	3928	*(IFT172)*	Intron
rs1260333	0.51	0.95	rs704795	5	2	27748624	20686565	5.55E-95	Triglycerides	Lipid level measurements	CVD risk factor (CVD RF);Lipids	05/08/2010	Nature	Biological, clinical and population relevance of 95 loci for blood lipids.	Up to 100184 EA individuals	9705 South Asians, 15046 East Asians, 8061 African Americans, 7063 Europeans	Mixed	140059	100184		
rs7586601	0.76	1	rs704795	5	2	27584666	20686565	2.24E-52	Triglycerides	Lipid level measurements	CVD risk factor (CVD RF);Lipids	05/08/2010	Nature	Biological, clinical and population relevance of 95 loci for blood lipids.	Up to 100184 EA individuals	9705 South Asians, 15046 East Asians, 8061 African Americans, 7063 Europeans	Mixed	140059	100184		
rs1260333	0.51	0.95	rs704795	5	2	27748624	19060906	1.09E-23	Triglycerides	Lipid level measurements	CVD risk factor (CVD RF);Lipids	07/12/2008	Nat Genet	Common variants at 30 loci contribute to polygenic dyslipidemia.	19840 EA individuals	Up to 20623 EA individuals	European	40463	19840		
rs7586601	0.76	1	rs704795	5	2	27584666	19060906	2.60E-13	Triglycerides	Lipid level measurements	CVD risk factor (CVD RF);Lipids	07/12/2008	Nat Genet	Common variants at 30 loci contribute to polygenic dyslipidemia.	19840 EA individuals	Up to 20623 EA individuals	European	40463	19840		
rs1260333	0.51	0.95	rs704795	5	2	27748624	20864672	1.70E-19	Triglycerides	Lipid level measurements	CVD risk factor (CVD RF);Lipids	23/09/2010	Arterioscler Thromb Vasc Biol	Genetic variants influencing circulating lipid levels and risk of coronary artery disease.	Up to 17243 EA individuals	Up to 37774 EA individuals, up to 9665 Indian Asian individuals	Mixed	64682	17243		

Query SNP	r2	D'	Meno. SNP	Meno. region	chr	pos(hg19)	PMID	P-value	Phenotype	Phenotype Description	Phenotype Categories	Date of Pub.	Journal	Title	Initial Sample Description	Replication Sample Description	GWAS ancestry Description	Total Samples (discovery + replication)	Total Discovery Samples	Suggested Gene	Function
rs4665972	0.51	1	rs704795	5	2	27598097	23726366	1.05E-08	Triglycerides	Lipid level measurements	CVD risk factor (CVD RF);Lipids	30/05/2013	Am J Hum Genet	Genome-wide characterization of shared and distinct genetic components that influence blood lipid levels in ethnically diverse human populations.	7,917 African American individuals, 3,506 Hispanic individuals	7,138 African American individuals	Mixed	18561	11423	*(SNX17)*	Intron
rs7586601	0.76	1	rs704795	5	2	27584666	23063622	3.72E-37	Triglycerides	Lipid level measurements	CVD risk factor (CVD RF);Lipids	11/10/2012	Am J Hum Genet	Large-scale gene-centric meta-analysis across 32 studies identifies multiple lipid loci.	66,240 individuals	25,282 individuals	European	91522	66240		
rs1260333	0.51	0.95	rs704795	5	2	27748624	20339536	1.40E-11	Triglycerides change with statins	Response to statin treatment (simvastatin, pravastatin, atorvastatin), change in cholesterol levels	Drug response;Quantitative trait(s);CVD risk factor (CVD RF);Lipids	22/03/2010	PLoS One	Genome-wide association of lipid-lowering response to statins in combined study populations.	3928 EA individuals	NR	European	3928	3928		
rs780110	0.82	1	rs704795	5	2	27685388	20339536	4.30E-08	Triglycerides change with statins	Response to statin treatment (simvastatin, pravastatin, atorvastatin), change in cholesterol levels	Drug response;Quantitative trait(s);CVD risk factor (CVD RF);Lipids	22/03/2010	PLoS One	Genome-wide association of lipid-lowering response to statins in combined study populations.	3928 EA individuals	NR	European	3928	3928	*(IFT172)*	Intron
rs1260333	0.51	0.95	rs704795	5	2	27748624	19802338	7.20E-10	Triglycerides, ln	Lipid level measurements, in plasma	CVD risk factor (CVD RF);Lipids;Plasma;Gender;Male;Female	01/10/2008	Circ Cardiovasc Genet	Genetic loci associated with plasma concentration of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, apolipoprotein A1, and Apolipoprotein B among 6382 white women in genome-wide analysis with replication.	6382 EA women	970 EA men and women	European	7352	6382		
rs1260333	0.51	0.95	rs704795	5	2	27748624	19503597	3.19E-08	Uric acid	Uric acid levels, in serum	Quantitative trait(s);Blood-related;Serum	05/06/2009	PLoS Genet	Meta-analysis of 28,141 individuals identifies common variants within five new loci that influence uric acid concentrations.	12328 European males, 15813 European females	NR	European	28141	28141		
rs1494961	0.94	1	rs4693089	9	4	84374480	21437268	1.00E-08	Upper aero-digestive tract cancer	Upper airway tract cancer	Cancer;Upper airway tract cancer	17/03/2011	PLoS Genet	A genome-wide association study of upper aerodigestive tract cancers conducted within the INHANCE consortium.	2091 EA cases, 8334 EA controls	8744 EA cases, 11982 EA controls	European	31151	10425	*(HELQ)*	Missense
rs12207488	0.96	1	rs9393800	14b	6	10952336	21829377	4.87E-16	Plasma docosapentaenoic acid levels	Long chain n-3 polyunsaturated fatty acid levels, in plasma	Quantitative trait(s);Blood-related;Plasma	28/07/2011	PLoS Genet	Genetic Loci Associated with Plasma Phospholipid n-3 Fatty Acids: A Meta-Analysis of Genome-Wide Association Studies from the CHARGE Consortium.	8866 EA individuals	NR	European	8866	8866	*(SYCP2L)*	Intron

Query SNP	r2	D'	Meno. SNP	Meno. region	chr	pos(hg19)	PMID	P-value	Phenotype	Phenotype Description	Phenotype Categories	Date of Pub.	Journal	Title	Initial Sample Description	Replication Sample Description	GWAS ancestry Description	Total Samples (discovery + replication)	Total Discovery Samples	Suggested Gene	Function
rs4237036	0.6	1	rs10957156	18	8	61701057	23396134	1.52E-08	Refractive error	Myopia and refractive errors	Eye-related;Developmental	10/02/2013	Nat Genet	Genome-wide meta-analyses of multiancestry cohorts identify multiple new susceptibility loci for refractive error and myopia.	37,382 European ancestry individuals, 578 Han Chinese ancestry individuals, 3,417 Singaporean Chinese ancestry individuals, 2,273 Singaporean Malay ancestry individuals, 2,108 Singaporean Indian ancestry individuals	NR	Mixed	45758	45758	*(CHD7)*	Intron
rs883263	0.54	0.8	rs1727326	26	12	123485188	20881960	1.54E-16	Gene expression	Height	Quantitative trait(s);Height	29/09/2010	Nature	Hundreds of variants clustered in genomic loci and biological pathways affect human height.	133653 EA individuals	50074 EA individuals	European	183727	133653	*(PITPNM2)*	Intron
rs2928148	0.87	0.96	rs9796	30	15	41401550	22479191	4.00E-08	Serum creatinine estimated glomerular filtration rate (eGFR)	Chronic kidney disease (CKD)	Renal;Chronic kidney disease;Quantitative trait(s)	29/03/2012	PLoS Genet	Genome-wide association and functional follow-up reveals new loci for kidney function.	74,354 European ancestry individuals	56,246 European ancestry individuals	European	130600	74354	*(INO80)*	Intron
rs2928148	0.87	0.96	rs9796	30	15	41401550	22479191	3.70E-08	Serum creatinine estimated glomerular filtration rate (eGFR) without diabetes	Chronic kidney disease (CKD)	Renal;Chronic kidney disease;Quantitative trait(s)	29/03/2012	PLoS Genet	Genome-wide association and functional follow-up reveals new loci for kidney function.	74,354 European ancestry individuals	56,246 European ancestry individuals	European	130600	74354	*(INO80)*	Intron
rs10852344	1	1	rs10852344	33	16	12016919	23307926	4.83E-08	Menopause with early age of onset (<45 years)	Age at menopause (early menopause)	Developmental;Aging;Reproductive;Gender;Female;Menopause	21/01/2013	Hum Mol Genet	A genome-wide association study of early menopause and the combined impact of identified variants.	3,493 European ancestry cases, 13,598 European ancestry controls	3,412 European ancestry cases, 4,928 European ancestry controls	European	25431	17091		
rs2271308	0.55	0.89	rs2941505	36	17	37817482	21907864	1.70E-10	Asthma	Asthma	Asthma;Pulmonary;Chronic lung disease;Inflammation	10/09/2011	Lancet	Identification of IL6R and chromosome 11q13.5 as risk loci for asthma.	2669 EA asthmatics, 4528 controls	15797 EA asthmatics, 42003 controls (pooled)	European	64997	7197	*(STARD3)*	Intron
rs2941504	0.92	1	rs2941505	36	17	37830900	20860503	8.30E-09	Asthma, childhood and later onset	Asthma	Asthma;Pulmonary;Chronic lung disease;Inflammation	23/09/2010	N Engl J Med	A large-scale, consortium-based genomewide association study of asthma.	10365 EA cases, 16110 controls	NR	European	26475	26475	*(PGAP3)*	Synonymous
rs11869286	0.69	0.83	rs2941505	36	17	37813856	20686565	3.00E-18	CRKRS gene expression in human omental fat	Lipid level measurements	CVD risk factor (CVD RF);Lipids	05/08/2010	Nature	Biological, clinical and population relevance of 95 loci for blood lipids.	Up to 100184 EA individuals	9705 South Asians, 15046 East Asians, 8061 African Americans, 7063 Europeans	Mixed	140059	100184	*(STARD3)*	Intron
rs1053651	0.51	0.83	rs2941505	36	17	37822311	23222517	3.78E-08	Red blood cell count (RBC)	Blood cell traits, in red blood cells	Quantitative trait(s);Blood-related	20/12/2012	Nature	Seventy-five genetic loci influencing the human red blood cell.	62,553 European ancestry individuals, 9,308 South Asian ancestry individuals	63,506 European ancestry individuals	Mixed	135367	71861	*(TCAP)*	Synonymous

Table S17. Genetic correlations across a range of phenotypes using the Broad Group Method.

Phenotype	Reg. Coef.	SE	Z	p
age at onset of menopause	0.983	0.012	83.83	< 1E-299
obesity class 1	-0.154	0.044	-3.512	4.447E-04
BMI in women	-0.165	0.053	-3.131	1.742E-03
age at onset of menarche	0.136	0.046	2.968	2.997E-03
BMI	-0.130	0.044	-2.949	3.188E-03
waist circumference in women	-0.164	0.063	-2.609	9.081E-03
extreme BMI	-0.164	0.064	-2.563	0.010
overweight	-0.115	0.046	-2.502	0.012
HDL	0.138	0.059	2.35	0.019
WHR in men	-0.200	0.093	-2.157	0.031
current/former smokers	0.198	0.095	2.078	0.038
femoral neck bone mineral density in women	0.136	0.070	1.936	0.053
obesity class 1	-0.104	0.056	-1.848	0.065
anorexia	0.121	0.066	1.835	0.067
years of education	0.107	0.059	1.801	0.072
ln(HOMA-IR)	-0.210	0.119	-1.774	0.076
obesity class 3	-0.149	0.086	-1.734	0.083
college	0.101	0.061	1.654	0.098
hip circumference in women	-0.117	0.074	-1.581	0.114
ever/never smokers	-0.106	0.069	-1.524	0.128
lumbar spine bone mineral density in women	0.102	0.072	1.419	0.156
BMI in men	-0.078	0.058	-1.357	0.175
cigarettes per day	-0.134	0.104	-1.286	0.198
WHR in women	-0.089	0.080	-1.112	0.266
weight in men	-0.060	0.056	-1.061	0.289
waist circumference in men	-0.082	0.078	-1.049	0.294
HbA1c	-0.078	0.083	-0.9413	0.347
femoral neck bone mineral density	0.056	0.060	0.9255	0.355
ln(HOMA-B)	-0.113	0.123	-0.9166	0.359
fasting glucose	-0.076	0.086	-0.8844	0.376
bipolar	0.061	0.069	0.8743	0.382
triglycerides	-0.055	0.064	-0.8519	0.394
lumbar spine bone mineral density	0.056	0.066	0.8483	0.396
childhood obesity	-0.052	0.073	-0.7116	0.477
Alzheimers	0.055	0.085	0.6475	0.517
birth weight	0.054	0.090	0.6057	0.545
height in women	0.028	0.049	0.5738	0.566
lumbar spine bone mineral density in men	-0.052	0.093	-0.5614	0.575
total cholesterol	0.026	0.061	0.4224	0.673
birth length	-0.032	0.087	-0.3652	0.715
ln(fasting proinsulin)	-0.048	0.169	-0.2851	0.776
LDL	0.016	0.060	0.2674	0.789
femoral neck bone mineral density in men	0.023	0.102	0.2292	0.819
hip circumference in men	0.019	0.090	0.2087	0.835
log(age at onset of smoking)	0.028	0.138	0.2035	0.839
rheumatoid arthritis	-0.016	0.094	-0.1661	0.868
asthma	0.017	0.107	0.1596	0.873
T2D	-0.012	0.078	-0.1579	0.875
major depression	0.015	0.106	0.1455	0.884
height	0.006	0.042	0.1452	0.885
WHR	0.013	0.092	0.1364	0.892
extreme height	0.007	0.050	0.1298	0.897
height in men	-0.006	0.052	-0.1075	0.914
infant head circumference	0.007	0.096	0.06904	0.945

Table S18. Details of the BMI to age at menopause score analysis.

BMI associated SNPs	BMI data				Menopause data	
MarkerName	Effect allele	Other Allele	Effect	SE	Aligned Effect	SE
rs10150332	C	T	0.13	0.03	0.05	0.03
rs10767664	A	T	0.19	0.03	-0.03	0.03
rs10938397	G	A	0.18	0.02	0.00	0.02
rs10968576	G	A	0.11	0.02	0.04	0.02
rs11847697	T	C	0.17	0.05	-0.01	0.06
rs12444979	C	T	0.17	0.03	0.01	0.03
rs13078807	G	A	0.1	0.02	-0.03	0.03
rs13107325	T	C	0.19	0.04	-0.10	0.04
rs1514175	A	G	0.07	0.02	0.00	0.02
rs1555543	C	A	0.06	0.02	-0.08	0.02
rs1558902	A	T	0.39	0.02	-0.01	0.02
rs206936	G	A	0.06	0.02	0.01	0.03
rs2112347	T	G	0.1	0.02	0.04	0.02
rs2241423	G	A	0.13	0.02	-0.02	0.02
rs2287019	C	T	0.15	0.03	-0.02	0.03
rs2815752	A	G	0.13	0.02	0.02	0.02
rs2867125	C	T	0.31	0.03	-0.04	0.03
rs2890652	C	T	0.09	0.03	0.05	0.03
rs29941	G	A	0.06	0.02	0.01	0.02
rs3810291	A	G	0.09	0.02	0.00	0.02
rs3817334	T	C	0.06	0.02	0.00	0.02
rs4771122	G	A	0.09	0.03	0.05	0.02
rs4836133	A	C	0.07	0.02	0.02	0.02
rs4929949	C	T	0.06	0.02	-0.02	0.02
rs543874	G	A	0.22	0.03	0.00	0.03
rs571312	A	C	0.23	0.03	0.00	0.02
rs713586	C	T	0.14	0.02	-0.02	0.02
rs7138803	A	G	0.12	0.02	0.05	0.02
rs7359397	T	C	0.15	0.02	-0.02	0.02
rs887912	T	C	0.1	0.02	0.02	0.02
rs9816226	T	A	0.14	0.03	-0.03	0.03
rs987237	G	A	0.13	0.03	0.05	0.03

Table S19. Details of the binomial analysis of directional consistency of age at menopause SNPs on BMI.

SNP	Meno. data		BMI increasing meno increasing?
	Effect	Other	
rs1054875	A	T	Yes
rs10734411	A	G	No
rs10852344	T	C	Yes
rs10905065	A	G	No
rs10957156	A	G	Yes
rs11031006	A	G	No
rs11668344	A	G	No
rs11738223	A	G	No
rs1183272	T	C	Yes
rs12142240	T	C	Yes
rs12196873	A	C	No
rs12461110	A	G	No
rs12599106	A	T	Yes
rs12824058	A	G	No
rs13040088	A	G	No
rs1411478	A	G	No
rs16858210	A	G	No
rs16991615	A	G	Yes
rs1713460	A	G	No
rs1727326	C	G	Yes
rs1799949	A	G	No
rs1800932	A	G	Yes
rs2230365	T	C	Yes
rs2236553	T	C	No
rs2236918	C	G	Yes
rs2241584	A	G	No
rs2277339	T	G	Yes
rs2547274	C	G	Yes
rs2720044	A	C	Yes
rs2941505	A	G	No
rs349306	A	G	Yes
rs365132	T	G	No
rs3741604	T	C	Yes
rs4246511	T	C	Yes
rs427394	A	G	No
rs451417	A	C	Yes
rs4693089	A	G	Yes
rs4879656	A	C	Yes
rs4886238	A	G	Yes
rs551087	A	G	No
rs5762534	T	C	No
rs6484478	A	G	No
rs6856693	A	G	Yes
rs6899676	A	G	Yes
rs704795	A	G	Yes
rs707938	A	G	No
rs7259376	A	G	Yes
rs7397861	C	G	Yes
rs763121	A	G	Yes
rs8070740	A	G	No
rs9039	T	C	No
rs930036	A	G	Yes
rs9393800	A	G	No
rs9796	A	T	Yes

Binomial tes	
Consistent	29
Divergent	25
Binomial p	0.683

Table S20. Details of the age at menarche to age at menopause score analysis.

SNP	Effect Allele	Other Allele	Age at menarche		Menopause	
			Effect	SE	Effect	SE
rs10144321	A	G	0.04	0.006	-0.014	0.024
rs1038903	T	C	0.04	0.006	0.014	0.023
rs10423674	A	C	0.04	0.005	0.005	0.022
rs10453225	G	T	0.09	0.005	0.025	0.022
rs10739221	C	T	0.08	0.006	0.017	0.024
rs10789181	A	G	0.03	0.005	0.023	0.021
rs1079866	G	C	0.07	0.007	0.020	0.030
rs10816359	T	G	0.04	0.008	0.018	0.030
rs10895140	G	A	0.04	0.005	-0.022	0.021
rs10938397	A	G	0.04	0.005	0.000	0.021
rs10980854	A	G	0.06	0.011	-0.011	0.045
rs10980921	C	T	0.09	0.009	0.021	0.036
rs11022756	A	C	0.05	0.006	0.018	0.023
rs11165924	A	G	0.03	0.006	-0.019	0.023
rs11215400	C	A	0.04	0.006	-0.017	0.024
rs1129700	T	C	0.03	0.005	-0.004	0.023
rs11578152	G	A	0.03	0.005	0.032	0.020
rs11715566	T	C	0.05	0.005	0.006	0.020
rs11767400	A	C	0.04	0.006	-0.010	0.023
rs11792861	A	C	0.04	0.005	-0.030	0.022
rs12148769	G	A	0.05	0.008	0.071	0.035
rs12446632	A	G	0.04	0.007	-0.006	0.030
rs12472911	C	T	0.04	0.006	0.006	0.026
rs1254337	T	A	0.04	0.005	-0.026	0.022
rs12571664	T	C	0.04	0.006	-0.074	0.026
rs12607903	C	T	0.04	0.005	0.013	0.023
rs12915845	C	T	0.03	0.005	-0.029	0.021
rs13053505	G	T	0.04	0.007	0.047	0.027
rs13067731	T	C	0.04	0.007	-0.001	0.028
rs13135934	C	G	0.03	0.005	-0.021	0.021
rs13179411	T	G	0.06	0.007	0.011	0.028
rs13196561	C	A	0.04	0.006	-0.053	0.026
rs1324913	G	T	0.03	0.005	-0.010	0.021
rs1364063	C	T	0.05	0.005	-0.020	0.020
rs1400974	A	G	0.05	0.005	-0.015	0.021
rs1461503	C	A	0.05	0.005	-0.009	0.020
rs1469039	A	G	0.05	0.007	-0.003	0.027
rs1532331	G	T	0.03	0.005	-0.031	0.023
rs16860328	G	A	0.04	0.005	0.011	0.020
rs16896742	G	A	0.04	0.006	-0.005	0.027
rs16918254	A	G	0.05	0.009	-0.020	0.040
rs16918636	T	C	0.03	0.006	-0.007	0.025
rs17086188	A	G	0.07	0.013	0.086	0.058
rs17171818	C	T	0.04	0.006	0.008	0.024
rs17233066	C	T	0.09	0.014	-0.021	0.067
rs17236969	T	C	0.05	0.008	0.024	0.032
rs17266097	T	C	0.04	0.005	0.034	0.021
rs1874984	C	G	0.04	0.005	0.017	0.022
rs1915146	G	A	0.03	0.005	-0.036	0.021
rs1958560	A	G	0.03	0.005	-0.040	0.021
rs2063730	C	A	0.05	0.007	0.119	0.027
rs2137289	A	G	0.05	0.005	0.009	0.021
rs2153127	T	C	0.08	0.005	0.033	0.020
rs2274465	C	G	0.03	0.005	0.007	0.021
rs239198	T	C	0.03	0.005	0.002	0.021
rs244293	G	A	0.03	0.005	0.049	0.021
rs246185	C	T	0.04	0.006	-0.031	0.022
rs2479724	T	C	0.03	0.005	-0.005	0.020
rs251130	G	A	0.04	0.006	0.027	0.023
rs2600959	A	G	0.04	0.005	0.029	0.022
rs268067	A	G	0.04	0.006	0.020	0.026
rs2687729	G	A	0.04	0.006	-0.004	0.023

SNP	Effect Allele	Other Allele	Age at menarche		Menopause	
			Effect	SE	Effect	SE
rs2688325	T	C	0.03	0.006	0.014	0.023
rs2836950	C	G	0.03	0.005	0.003	0.022
rs2947411	A	G	0.06	0.007	0.047	0.027
rs3101336	T	C	0.04	0.005	-0.016	0.021
rs3733631	C	G	0.05	0.007	0.012	0.028
rs3743266	T	C	0.04	0.005	0.021	0.022
rs4369815	T	G	0.06	0.01	0.097	0.041
rs466639	C	T	0.08	0.007	0.034	0.031
rs4756059	T	C	0.07	0.01	-0.031	0.039
rs4840086	A	G	0.04	0.005	0.018	0.020
rs4875053	G	C	0.03	0.006	0.029	0.040
rs4895808	C	T	0.03	0.005	0.040	0.020
rs4929947	G	C	0.04	0.005	0.034	0.021
rs4946632	C	T	0.01	0.01	0.040	0.036
rs543874	A	G	0.05	0.006	0.001	0.026
rs6009583	C	T	0.03	0.006	0.023	0.024
rs6427782	A	G	0.03	0.005	0.065	0.021
rs652260	T	C	0.03	0.005	0.034	0.021
rs6555855	G	A	0.04	0.006	-0.003	0.025
rs6563739	G	T	0.03	0.005	-0.021	0.021
rs6747380	A	G	0.07	0.007	-0.018	0.027
rs6758290	T	C	0.04	0.005	0.018	0.021
rs6762477	G	A	0.04	0.006	-0.015	0.036
rs6770162	A	G	0.04	0.005	0.017	0.021
rs6933660	C	A	0.03	0.005	-0.019	0.022
rs6938574	T	C	0.04	0.007	-0.007	0.029
rs6964833	T	C	0.04	0.006	-0.019	0.024
rs7037266	A	C	0.03	0.005	-0.029	0.021
rs7103411	C	T	0.04	0.006	0.032	0.025
rs7104764	G	A	0.03	0.006	-0.017	0.024
rs7138803	G	A	0.04	0.005	-0.047	0.021
rs7141210	T	C	0.03	0.005	0.039	0.022
rs7215990	G	A	0.04	0.006	-0.008	0.025
rs7463166	A	G	0.03	0.005	0.031	0.021
rs7514705	C	T	0.04	0.005	0.007	0.021
rs7642134	G	A	0.04	0.005	-0.004	0.021
rs7647973	A	G	0.05	0.006	0.039	0.023
rs7701886	A	G	0.03	0.005	0.006	0.021
rs7759938	C	T	0.12	0.005	0.047	0.022
rs7821178	C	A	0.04	0.005	0.009	0.022
rs7828501	G	A	0.04	0.005	0.021	0.020
rs7853970	T	C	0.03	0.005	-0.021	0.022
rs7865468	A	G	0.03	0.005	0.009	0.022
rs7955374	T	C	0.04	0.008	0.025	0.032
rs8032675	T	C	0.04	0.005	0.012	0.020
rs8050136	C	A	0.04	0.005	0.009	0.021
rs852069	G	A	0.04	0.005	0.017	0.021
rs889122	G	T	0.04	0.006	0.006	0.023
rs900400	T	C	0.03	0.005	0.024	0.021
rs913588	G	A	0.03	0.005	-0.015	0.020
rs9321659	A	G	0.06	0.008	-0.043	0.031
rs939317	G	A	0.04	0.006	0.010	0.024
rs9447700	C	T	0.03	0.005	-0.009	0.023
rs9475752	C	T	0.04	0.006	0.019	0.026
rs951366	T	C	0.03	0.005	-0.007	0.021
rs9560113	G	A	0.05	0.006	0.008	0.023
rs9635759	A	G	0.05	0.005	0.014	0.023
rs9647570	G	T	0.05	0.007	0.012	0.030
rs9849248	C	T	0.04	0.007	0.042	0.029
rs988913	C	T	0.04	0.005	0.014	0.021

Table S21. Details of the age at menopause to age at menarche score analysis.

SNP	Effect Allele	Other Allele	Menopause		Age at Menarche	
			Effect	SE	Effect	SE
rs1054875	A	T	0.188	0.021	0.001	0.006
rs10734411	A	G	-0.122	0.021	0.004	0.006
rs10852344	T	C	-0.165	0.021	0.000	0.006
rs10905065	A	G	-0.113	0.021	-0.002	0.006
rs10957156	A	G	-0.139	0.024	0.007	0.007
rs11031006	A	G	0.217	0.029	0.027	0.009
rs11668344	A	G	0.412	0.021	0.007	0.006
rs11738223	A	G	-0.123	0.022	-0.001	0.006
rs1183272	T	C	0.071	0.021	-0.001	0.006
rs12142240	T	C	-0.127	0.022	0.009	0.006
rs12196873	A	C	-0.162	0.029	-0.002	0.008
rs12461110	A	G	-0.174	0.022	0.009	0.006
rs12599106	A	T	-0.116	0.021	-0.008	0.006
rs12824058	A	G	0.136	0.021	-0.001	0.006
rs13040088	A	G	0.158	0.025	-0.009	0.007
rs1411478	A	G	-0.132	0.021	0.005	0.006
rs16858210	A	G	0.141	0.024	-0.010	0.007
rs16991615	A	G	0.875	0.044	-0.005	0.013
rs1713460	A	G	0.144	0.023	0.003	0.006
rs1727326	C	G	-0.195	0.032	-0.022	0.009
rs1799949	A	G	0.139	0.021	-0.007	0.006
rs1800932	A	G	-0.173	0.026	-0.018	0.008
rs2230365	T	C	0.175	0.028	-0.005	0.008
rs2236553	T	C	0.157	0.025	-0.001	0.007
rs2236918	C	G	-0.153	0.021	0.000	0.006
rs2241584	A	G	-0.139	0.021	-0.001	0.006
rs2277339	T	G	0.312	0.035	0.001	0.010
rs2547274	C	G	0.277	0.038	0.003	0.011
rs2720044	A	C	-0.290	0.030	0.000	0.009
rs2941505	A	G	-0.130	0.022	-0.002	0.006
rs349306	A	G	0.227	0.036	-0.024	0.011
rs365132	T	G	0.242	0.020	-0.009	0.006
rs3741604	T	C	-0.092	0.022	-0.001	0.006
rs4246511	T	C	0.218	0.023	0.006	0.007
rs427394	A	G	0.126	0.022	0.012	0.006
rs451417	A	C	-0.195	0.033	0.021	0.010
rs4693089	A	G	-0.202	0.021	-0.015	0.006
rs4879656	A	C	-0.119	0.021	0.003	0.006
rs4886238	A	G	0.177	0.022	0.001	0.006
rs551087	A	G	0.125	0.023	-0.019	0.007
rs5762534	T	C	-0.164	0.028	0.008	0.008
rs6484478	A	G	0.099	0.024	0.005	0.007
rs6856693	A	G	-0.163	0.021	0.004	0.006
rs6899676	A	G	-0.229	0.025	0.010	0.007
rs704795	A	G	-0.163	0.021	0.004	0.006
rs707938	A	G	0.169	0.022	0.015	0.006
rs7259376	A	G	-0.111	0.020	0.003	0.006
rs7397861	C	G	0.095	0.021	-0.001	0.006
rs763121	A	G	0.165	0.022	0.009	0.007
rs8070740	A	G	-0.147	0.024	0.016	0.007
rs9039	T	C	0.125	0.023	-0.016	0.006
rs930036	A	G	-0.185	0.021	0.009	0.006
rs9393800	A	G	0.169	0.023	-0.006	0.007
rs9796	A	T	0.131	0.020	0.002	0.006

Table S22. Puberty MAGENTA analysis of enrichment for puberty timing genes.

Custom pathway	Gene_Symbol	Entrez_ID	Gene_p-value	Chr	Gene_Start_Pos	Gene_End_Pos	Gene_Size_kb	Num_SNPs_per_Gene
Monogenic puberty	*KISS1R*	84634	1.85E-06	19	868341	872015	4	57
Monogenic puberty	*CHD7*	55636	5.25E-05	8	61753892	61942021	188	300
Monogenic puberty	*FGFR1*	2260	1.14E-03	8	38387812	38445509	58	96
Monogenic puberty	*TAC3*	6866	1.21E-02	12	55690050	55696592	7	75
Monogenic puberty	*BRWD2*	55717	2.48E-01	10	122600684	122659026	58	346
Monogenic puberty	*FGF8*	2253	2.85E-01	10	103519876	103525817	6	32
Monogenic puberty	*PROK2*	60675	2.87E-01	3	71903495	71917047	14	129
Monogenic puberty	*KISS1*	3814	2.87E-01	1	202426091	202432242	6	169
Monogenic puberty	*HS6ST1*	9394	4.64E-01	2	128739523	128792641	53	213
Monogenic puberty	*SOX10*	6663	5.19E-01	22	36698264	36710485	12	58
Monogenic puberty	*GNRH1*	2796	5.25E-01	8	25332690	25338473	6	140
Monogenic puberty	*SEMA3A*	10371	6.46E-01	7	83425594	83662153	237	417
Monogenic puberty	*LEPR*	3953	6.68E-01	1	65658905	65875410	217	387
Monogenic puberty	*MKRN3*	7681	7.28E-01	15	21361546	21364259	3	103
Monogenic puberty	*LEP*	3952	7.62E-01	7	127668566	127684918	16	117
Monogenic puberty	*GNRHR*	2798	7.98E-01	4	68285693	68304399	19	146
Monogenic puberty	*PROKR2*	128674	8.38E-01	20	5230685	5243015	12	258
Monogenic puberty	*TACR3*	6870	8.48E-01	4	104730073	104860422	130	225
Monogenic puberty	*NELF*	26012	9.67E-01	9	139462497	139473575	11	19
Monogenic puberty	*NR0B1*	190	NaN	23	30232459	30237416	5	0
Monogenic puberty	*KAL1*	3730	NaN	23	8456914	8660227	203	0
Puberty (polygenic)	*FSHB*	2488	4.85E-10	11	30209138	30213400	4	156
Puberty (polygenic)	*PGR*	5241	2.72E-04	11	100405564	100505754	100	321
Puberty (polygenic)	*IL20RB*	53833	2.05E-03	3	138159396	138212610	53	145
Puberty (polygenic)	*GAB2*	9846	2.11E-03	11	77603989	77806414	202	259
Puberty (polygenic)	*CTBP2*	1488	9.92E-03	10	126666407	126839614	173	361
Puberty (polygenic)	*SEC23IP*	11196	2.34E-02	10	121642212	121691235	49	167
Puberty (polygenic)	*EEFSEC*	60678	3.26E-02	3	129355002	129610179	255	296
Puberty (polygenic)	*NPTXR*	23467	5.25E-02	22	37544402	37569963	26	136
Puberty (polygenic)	*SMARCAD1*	56916	6.63E-02	4	95347781	95431466	84	151
Puberty (polygenic)	*ACAD11*	84129	1.12E-01	3	133759671	133861665	102	113
Puberty (polygenic)	*PHF15*	23338	1.29E-01	5	133889696	133946817	57	133
Puberty (polygenic)	*TNNI3K*	51086	1.37E-01	1	74436534	74782696	346	382
Puberty (polygenic)	*DST*	667	1.43E-01	6	56430743	56816422	386	348
Puberty (polygenic)	*NEGR1*	257194	1.76E-01	1	71641212	72520865	880	735
Puberty (polygenic)	*ARNTL*	406	1.96E-01	11	13255900	13365388	109	321
Puberty (polygenic)	*CCNL1*	57018	1.97E-01	3	158348279	158361176	13	119
Puberty (polygenic)	*NFAT5*	10725	1.98E-01	16	68156497	68296054	140	156
Puberty (polygenic)	*PCDH7*	5099	2.02E-01	4	30331134	30757519	426	458
Puberty (polygenic)	*LEKR1*	389170	2.10E-01	3	158026789	158246612	220	322
Puberty (polygenic)	*TMEM38B*	55151	2.17E-01	9	107496645	107577265	81	183
Puberty (polygenic)	*KCNK9*	51305	2.18E-01	8	140693985	140784481	90	295
Puberty (polygenic)	*TBX6*	6911	2.21E-01	16	30004582	30010709	6	30
Puberty (polygenic)	*C6orf65*	221336	2.46E-01	6	56927731	57000101	72	118
Puberty (polygenic)	*NR5A2*	2494	2.55E-01	1	198263392	198413173	150	291
Puberty (polygenic)	*TCF7*	6932	2.56E-01	5	133478300	133511819	34	135
Puberty (polygenic)	*FRS3*	10817	2.58E-01	6	41845891	41855608	10	74
Puberty (polygenic)	*ALOX15B*	247	2.65E-01	17	7883082	7893176	10	113
Puberty (polygenic)	*EVI5L*	115704	2.76E-01	19	7801242	7835862	35	104
Puberty (polygenic)	*IGSF11*	152404	2.92E-01	3	120102168	120347588	245	310
Puberty (polygenic)	*BCDIN3D*	144233	2.93E-01	12	48517824	48523142	5	115
Puberty (polygenic)	*PCSK2*	5126	3.08E-01	20	17155630	17413222	258	537
Puberty (polygenic)	*COG4*	25839	3.22E-01	16	69071972	69114958	43	56
Puberty (polygenic)	*GPR45*	11250	3.29E-01	2	105224631	105226356	2	144
Puberty (polygenic)	*SCRIB*	23513	3.29E-01	8	144945077	144969537	24	59
Puberty (polygenic)	*INHBA*	3624	3.34E-01	7	41695125	41709231	14	143
Puberty (polygenic)	*CBX7*	23492	3.34E-01	22	37856724	37878484	22	144
Puberty (polygenic)	*FUT8*	2530	3.36E-01	14	64947592	65279715	332	376
Puberty (polygenic)	*EIF4G1*	1981	3.54E-01	3	185515049	185535840	21	72
Puberty (polygenic)	*SEC16B*	89866	3.55E-01	1	176164864	176205673	41	212
Puberty (polygenic)	*UBA7*	7318	3.64E-01	3	49817641	49826395	9	59
Puberty (polygenic)	*DPYD*	1806	3.67E-01	1	97315887	98159203	843	697
Puberty (polygenic)	*SIM1*	6492	3.67E-01	6	100943470	101018272	75	192
Puberty (polygenic)	*VDR*	7421	3.74E-01	12	46521586	46585081	63	220
Puberty (polygenic)	*KCTD13*	253980	3.77E-01	16	29825161	29845046	20	58
Puberty (polygenic)	*NUCKS1*	64710	3.90E-01	1	203948569	203985984	37	155
Puberty (polygenic)	*GALNT10*	55568	3.99E-01	5	153550487	153780003	230	526
Puberty (polygenic)	*NTRK2*	4915	4.00E-01	9	86473285	86828325	355	590
Puberty (polygenic)	*DET1*	55070	4.01E-01	15	86856717	86890888	34	151
Puberty (polygenic)	*MKL2*	57496	4.21E-01	16	14072696	14268131	195	239
Puberty (polygenic)	*C13orf16*	121793	4.23E-01	13	110771015	110794595	24	163
Puberty (polygenic)	*TYW3*	127253	4.24E-01	1	74971427	75003896	32	152
Puberty (polygenic)	*BYSL*	705	4.24E-01	6	41996942	42008762	12	82
Puberty (polygenic)	*FAM83B*	222584	4.31E-01	6	54819527	54914778	95	353
Puberty (polygenic)	*GTF2I*	2969	4.37E-01	7	73709965	73812958	103	32
Puberty (polygenic)	*WWP2*	11060	4.38E-01	16	68353774	68533144	179	180
Puberty (polygenic)	*COG6*	57511	4.38E-01	13	39127813	39224633	97	267
Puberty (polygenic)	*DLK1*	8788	4.42E-01	14	100263005	100271212	8	166
Puberty (polygenic)	*C9orf5*	23731	4.49E-01	9	110817235	110922046	105	306
Puberty (polygenic)	*THRB*	7068	4.59E-01	3	24133648	24511317	378	550

Custom pathway	Gene_Symbol	Entrez_ID	Gene_p-value	Chr	Gene_Start_Pos	Gene_End_Pos	Gene_Size_kb	Num_SNPs_per_Gene
Puberty (polygenic)	*ESR1*	2099	4.64E-01	6	152053323	152466101	413	617
Puberty (polygenic)	*JMJD2C*	23081	4.71E-01	9	6747653	7165648	418	890
Puberty (polygenic)	*BDNF*	627	4.74E-01	11	27633017	27699872	67	147
Puberty (polygenic)	*ASCC3*	10973	4.77E-01	6	101063328	101435945	373	407
Puberty (polygenic)	*SLIT3*	6586	4.81E-01	5	168025648	168660711	635	970
Puberty (polygenic)	*OLFM3*	118427	4.87E-01	1	102040714	102235378	195	398
Puberty (polygenic)	*ZNF483*	158399	4.88E-01	9	113327267	113379945	53	130
Puberty (polygenic)	*HCRTR2*	3062	4.88E-01	6	55147029	55255377	108	229
Puberty (polygenic)	*TMEM18*	129787	4.92E-01	2	657972	667439	9	235
Puberty (polygenic)	*LGR4*	55366	4.92E-01	11	27344083	27450910	107	143
Puberty (polygenic)	*OLFM2*	93145	5.03E-01	19	9825393	9908070	83	116
Puberty (polygenic)	*ZNF654*	55279	5.07E-01	3	88270951	88276504	6	115
Puberty (polygenic)	*C6orf173*	387103	5.13E-01	6	126702970	126711441	8	67
Puberty (polygenic)	*CCDC85A*	114800	5.14E-01	2	56264761	56466813	202	436
Puberty (polygenic)	*LIN28B*	389421	5.15E-01	6	105511615	105637899	126	141
Puberty (polygenic)	*PARP10*	84875	5.19E-01	8	145123307	145132623	9	40
Puberty (polygenic)	*HTR1F*	3355	5.24E-01	3	88114415	88125609	11	99
Puberty (polygenic)	*RAB7L1*	8934	5.37E-01	1	204004300	204010747	6	116
Puberty (polygenic)	*BRWD1*	54014	5.45E-01	21	39479273	39607426	128	230
Puberty (polygenic)	*STXBP4*	252983	5.58E-01	17	50401124	50596448	195	353
Puberty (polygenic)	*NDN*	4692	5.64E-01	15	21481646	21483543	2	104
Puberty (polygenic)	*MAGEL2*	54551	5.64E-01	15	21439788	21442268	2	91
Puberty (polygenic)	*WSCD1*	23302	5.66E-01	17	5914657	5968471	54	215
Puberty (polygenic)	*NR4A2*	4929	5.67E-01	2	156889189	156897533	8	82
Puberty (polygenic)	*PXMP3*	5828	6.04E-01	8	78055048	78075079	20	150
Puberty (polygenic)	*TRPC6*	7225	6.07E-01	11	100827504	100959869	132	443
Puberty (polygenic)	*STARD4*	134429	6.09E-01	5	110861920	110876056	14	191
Puberty (polygenic)	*CADM1*	23705	6.17E-01	11	114549554	114880451	331	428
Puberty (polygenic)	*CADPS2*	93664	6.20E-01	7	121745713	122313790	568	610
Puberty (polygenic)	*RETN*	56729	6.32E-01	19	7639971	7641340	1	84
Puberty (polygenic)	*IMPG1*	3617	6.34E-01	6	76687781	76839055	151	214
Puberty (polygenic)	*IGF2BP2*	10644	6.36E-01	3	186844220	187025521	181	162
Puberty (polygenic)	*ZNF131*	7690	6.64E-01	5	43157398	43211580	54	106
Puberty (polygenic)	*LEPR*	3953	6.68E-01	1	65658905	65875410	217	387
Puberty (polygenic)	*RMI1*	80010	6.69E-01	9	85785456	85808807	23	73
Puberty (polygenic)	*PTPRK*	5796	6.74E-01	6	128331624	128883416	552	435
Puberty (polygenic)	*SFRS10*	6434	6.74E-01	3	187117228	187138450	21	113
Puberty (polygenic)	*PCSK1*	5122	6.80E-01	5	95751874	95794708	43	212
Puberty (polygenic)	*RORA*	6095	6.86E-01	15	58576754	59308794	732	1083
Puberty (polygenic)	*DLGAP1*	9229	6.87E-01	18	3488836	3870135	381	450
Puberty (polygenic)	*BCL11A*	53335	6.89E-01	2	60531805	60634137	102	179
Puberty (polygenic)	*CA10*	56934	6.99E-01	17	47062672	47592376	530	741
Puberty (polygenic)	*GHR*	2690	7.19E-01	5	42459782	42757683	298	291
Puberty (polygenic)	*NCOA7*	135112	7.27E-01	6	126153693	126293959	140	193
Puberty (polygenic)	*MKRN3*	7681	7.28E-01	15	21361546	21364259	3	103
Puberty (polygenic)	*THRSP*	7069	7.30E-01	11	77452554	77457045	4	139
Puberty (polygenic)	*SIX6*	4990	7.46E-01	14	60045690	60048278	3	121
Puberty (polygenic)	*RDH8*	50700	7.51E-01	19	9984924	9993954	9	99
Puberty (polygenic)	*ADARB2*	105	7.52E-01	10	1218072	1769718	552	956
Puberty (polygenic)	*JMJD1B*	51780	7.52E-01	5	137716183	137800615	84	117
Puberty (polygenic)	*FTO*	79068	7.74E-01	16	52295375	52705882	411	608
Puberty (polygenic)	*SIRT3*	23410	7.75E-01	11	205029	226362	21	144
Puberty (polygenic)	*ODZ2*	57451	7.85E-01	5	166644420	167623740	979	1079
Puberty (polygenic)	*BRD8*	10902	7.91E-01	5	137503357	137542257	39	54
Puberty (polygenic)	*RXRG*	6258	8.19E-01	1	163636973	163681054	44	274
Puberty (polygenic)	*NPBWR1*	2831	8.21E-01	8	54015020	54016007	1	129
Puberty (polygenic)	*KLF12*	11278	8.24E-01	13	73158149	73606067	448	709
Puberty (polygenic)	*POU1F1*	5449	8.43E-01	3	87391472	87408427	17	224
Puberty (polygenic)	*MAP2K5*	5607	8.44E-01	15	65622074	65886506	264	299
Puberty (polygenic)	*TACR3*	6870	8.48E-01	4	104730073	104860422	130	225
Puberty (polygenic)	*SATB2*	23314	8.48E-01	2	199842468	200033500	191	129
Puberty (polygenic)	*OR2K2*	26248	8.51E-01	9	113129583	113130534	1	120
Puberty (polygenic)	*PTPRF*	5792	8.57E-01	1	43769133	43861930	93	161
Puberty (polygenic)	*PTPRD*	5789	8.63E-01	9	8304245	10602509	2298	4150
Puberty (polygenic)	*PHF21A*	51317	8.73E-01	11	45907445	46099561	192	208
Puberty (polygenic)	*PTH*	5741	8.78E-01	11	13470176	13474143	4	108
Puberty (polygenic)	*LRP1B*	53353	8.80E-01	2	140705465	142605740	1900	2690
Puberty (polygenic)	*GNPDA2*	132789	8.83E-01	4	44398924	44423369	24	110
Puberty (polygenic)	*JMJD2A*	9682	8.87E-01	1	43888383	43943776	55	145
Puberty (polygenic)	*GPRC5B*	51704	8.97E-01	16	19777793	19803652	26	80
Puberty (polygenic)	*BSX*	390259	9.04E-01	11	122353566	122357589	4	142
Puberty (polygenic)	*WDR6*	11180	9.10E-01	3	49019829	49028386	9	34
Puberty (polygenic)	*CRTC1*	23373	9.12E-01	19	18655424	18754143	99	94
Puberty (polygenic)	*MCHR2*	84539	9.24E-01	6	100474506	100548835	74	139
Puberty (polygenic)	*CSMD1*	64478	9.26E-01	8	2780281	4839736	2059	5876

Table S23. Details of the breast cancer to age at menopause score analysis.

SNP	Effect Allele	Other Allele	Menopause		Breast Cancer	
			Effect	StdErr	Beta	SE
rs10069690	T	C	-0.1201	0.0586	0.058	0.010
rs1011970	T	G	-0.0121	0.0268	0.058	0.015
rs10472076	T	C	-0.011	0.0221	-0.049	0.010
rs10759243	A	C	0.0517	0.0238	0.058	0.015
rs10771399	A	G	-0.0455	0.0321	0.151	0.018
rs10941679	A	G	-0.0194	0.0239	-0.122	0.014
rs10995190	A	G	0.0265	0.028	-0.151	0.012
rs11199914	T	C	-0.0448	0.0226	-0.051	0.011
rs11242675	T	C	0.0277	0.021	0.062	0.011
rs11249433	A	G	-0.0223	0.0213	-0.086	0.009
rs11780156	T	C	0.018	0.0271	0.068	0.015
rs11814448	A	C	-0.045	0.0726	-0.231	0.033
rs11820646	T	C	0.0015	0.0207	-0.051	0.011
rs12422552	C	G	-0.0066	0.0242	0.049	0.010
rs12493607	C	G	0.0091	0.0215	0.058	0.015
rs1292011	A	G	-0.0218	0.0209	0.083	0.011
rs132390	T	C	-0.0651	0.0659	-0.113	0.023
rs13281615	A	G	0.0054	0.0206	-0.086	0.009
rs13329835	A	G	0.0077	0.0247	-0.077	0.014
rs13387042	A	G	-0.0092	0.0199	0.128	0.012
rs1353747	T	G	-0.0308	0.0347	0.083	0.017
rs1432679	T	C	-0.0204	0.0203	-0.068	0.010
rs1436904	T	G	0.0218	0.0206	0.041	0.011
rs1550623	A	G	-0.0051	0.0279	0.062	0.011
rs16857609	T	C	-0.0072	0.023	0.077	0.010
rs17356907	A	G	-0.0077	0.0244	0.094	0.011
rs17530068	T	C	0.0138	0.0238	-0.049	0.010
rs17817449	T	G	0.0098	0.0206	0.073	0.011
rs2016394	A	G	-0.0181	0.0206	-0.051	0.011
rs204247	A	G	0.023	0.0203	-0.049	0.010
rs2046210	A	G	-0.041	0.0213	0.077	0.010
rs2236007	A	G	0.0162	0.0267	-0.073	0.011
rs2588809	T	C	0.0023	0.0284	0.077	0.014
rs2823093	A	G	0.0163	0.023	-0.083	0.011
rs2943559	A	G	-0.0668	0.0385	-0.122	0.018
rs2981579	A	G	-0.0161	0.0206	0.239	0.012
rs3757318	A	G	-0.061	0.039	0.148	0.018
rs3760982	A	G	0.0018	0.0199	0.058	0.010
rs3803662	A	G	-0.0002	0.0227	0.215	0.012
rs3817198	T	C	-0.0124	0.0223	-0.068	0.010
rs3903072	T	G	-0.0258	0.0203	-0.051	0.011
rs4808801	A	G	0.0213	0.0213	0.073	0.011
rs4849887	T	C	-0.0337	0.0338	-0.094	0.017
rs4973768	T	C	0.0206	0.0201	-0.095	0.009
rs527616	C	G	-0.0154	0.0217	-0.051	0.011
rs6001930	T	C	-0.0629	0.0329	-0.113	0.014
rs614367	T	C	-0.0478	0.0295	0.191	0.013
rs616488	A	G	-0.0011	0.0216	0.062	0.011
rs6472903	T	G	-0.014	0.0273	0.094	0.011
rs6504950	A	G	0.0206	0.023	-0.062	0.011
rs6762644	A	G	0.0219	0.0207	-0.068	0.015
rs6828523	A	C	0.0189	0.0315	-0.105	0.017
rs704010	T	C	-0.0053	0.0208	0.077	0.010
rs7072776	A	G	0.0025	0.0227	0.068	0.010
rs720475	A	G	0.0452	0.0232	-0.062	0.011
rs7904519	A	G	-0.0036	0.0201	-0.058	0.010
rs8170	A	G	0.0086	0.0264	0.039	0.015
rs865686	T	G	0.0178	0.0209	0.117	0.006
rs889312	A	C	0.0107	0.0225	-0.113	0.009
rs941764	A	G	-0.0405	0.022	-0.058	0.010
rs9693444	A	C	-0.0098	0.0215	0.068	0.010
rs9790517	T	C	0.0114	0.0243	0.049	0.010
rs999737	T	C	0.0296	0.0238	-0.083	0.011

Table S24. Results from age stratified breast cancer analysis.

Quintile comparison	BC OR (CI) P-value	ER+ BC OR (CI) P-value	ER- BC OR (CI) P-value	Pre-menopausal* BC OR (CI) P-value	Post-menopausal* BC OR (CI) P-value
Per Quintile trend	1.03 (1.02-1.04) $3.2x10^{-12}$	1.04 (1.03-1.05) $2.3x10^{-10}$	1.02 (1.00-1.04) 0.017	1.00 (0.98-1.03) 0.97	1.03 (1.02-1.05) $1.1x10^{-6}$
Q5 vs Q1	1.13 (1.09-1.18) $8.4x10^{-9}$	1.15 (1.09-1.20) $1.14x10^{-7}$	1.10 (1.01-1.19) 0.026	1.00 (0.90-1.11) 0.98	1.14 (1.07-1.21) $5.2x10^{-5}$
Q1 vs Q3	0.94 (0.90-0.98) 0.0054	0.93 (0.89-0.98) 0.0077	0.99 (0.91-1.08) 0.84	0.97 (0.87-1.09) 0.65	0.95 (0.90-1.01) 0.13
Q5 vs Q3	1.07 (1.02-1.11) 0.0029	1.07 (1.02-1.12) 0.0083	1.09 (1.00-1.18) 0.042	0.97 (0.87-1.09) 0.63	1.08 (1.02-1.15) 0.010

Breast Cancer ORs by quintile of ANM PRS (quintiles defined in BC controls).

Pre-menopausal cases and controls are defined for these purposes as those with age≤45 years at diagnosis (cases) or interview (controls). Post-menopausal cases and controls are similarly defined as those with age ≥55 years at diagnosis (cases) or interview (controls).

	DDR SNPs	Non-DDR SNPs	Difference between DDR and non-DDR (permutation)
Pre-menopausal	1.00 (0.95-1.05) P=0.92	1.02 (0.94-1.15) P=0.74	0.77
Post-menopausal	1.04 (1.01-1.08) P=0.0017	1.14 (1.07-1.27) P=3.4x10-7	0.002
Difference between pre- and pos	P=0.0088	P=4.8x10-5	

Polygenic risk score of DDR vs non-DDR linked SNPs in the PRS

Table S25. Details of the menopause score to prostate cancer analysis.

SNP	Effect Allele	Other Allele	Menopause		Prostate cancer	
			Effect	SE	Effect	SE
rs1054875	a	t	0.1881	0.0208	0.005009	0.013998
rs10734411	a	g	-0.1221	0.0205	-0.01248	0.013745
rs10852344	t	c	-0.1646	0.0206	-0.00944	0.014111
rs10905065	a	g	-0.1128	0.0205	0.003618	0.013907
rs10957156	a	g	-0.1387	0.0237	0.028007	0.016454
rs11031006	a	g	0.2165	0.029	0.005488	0.019179
rs11668344	a	g	0.4115	0.0211	-0.0263	0.014383
rs11738223	a	g	-0.1234	0.022	0.012149	0.01458
rs1183272	t	c	0.0705	0.0209	0.012246	0.0149
rs12142240	t	c	-0.1267	0.0218	-0.0539	0.014675
rs12196873	a	c	-0.1618	0.0291	0.004927	0.019704
rs12461110	a	g	-0.1744	0.0216	0.002489	0.015195
rs12599106	a	t	-0.1159	0.0209	-0.01193	0.013741
rs12824058	a	g	0.1355	0.0207	-0.0125	0.01383
rs13040088	a	g	0.1575	0.0249	0.00142	0.016816
rs1411478	a	g	-0.1316	0.0205	0.015695	0.014052
rs16858210	a	g	0.1412	0.0238	0.029825	0.015613
rs16991615	a	g	0.8752	0.0436	0.008357	0.028895
rs1713460	a	g	0.144	0.0227	-0.00433	0.014852
rs1727326	c	g	-0.1948	0.0323	-0.04504	0.02234
rs1799949	a	g	0.1393	0.0214	-0.01499	0.014512
rs1800932	a	g	-0.1731	0.0261	0.025438	0.017904
rs2230365	t	c	0.1748	0.0284	0.033968	0.018795
rs2236553	t	c	0.1574	0.0254	-0.00763	0.017389
rs2236918	c	g	-0.1529	0.0205	0.014109	0.013735
rs2241584	a	g	-0.1394	0.0207	0.009764	0.014034
rs2277339	t	g	0.312	0.0346	-0.00458	0.022179
rs2547274	c	g	0.2765	0.038	0.020833	0.02298
rs2720044	a	c	-0.29	0.0302	-0.02539	0.01832
rs2941505	a	g	-0.1303	0.0217	0.026823	0.014613
rs349306	a	g	0.2273	0.0356	0.021773	0.019579
rs365132	t	g	0.2424	0.0201	0.016597	0.013558
rs3741604	t	c	-0.0918	0.0215	0.011084	0.01704
rs4246511	t	c	0.2181	0.0232	0.001268	0.014694
rs427394	a	g	0.1264	0.0215	0.004892	0.01675
rs451417	a	c	-0.1954	0.0333	-0.03457	0.020634
rs4693089	a	g	-0.2022	0.0206	0.011537	0.013734
rs4879656	a	c	-0.1188	0.0212	0.015203	0.014152
rs4886238	a	g	0.1768	0.0216	0.016332	0.014491
rs551087	a	g	0.1254	0.0228	-0.01704	0.015143
rs5762534	t	c	-0.1636	0.0281	-0.03728	0.018786
rs6484478	a	g	0.0992	0.0241	0.001043	0.016543
rs6856693	a	g	-0.1626	0.021	0.008982	0.01419
rs6899676	a	g	-0.2289	0.0254	-0.01545	0.017033
rs704795	a	g	-0.1634	0.0206	-0.01545	0.013858
rs707938	a	g	0.1691	0.0217	0.039269	0.014599
rs7259376	a	g	-0.1106	0.0202	-0.01389	0.013872
rs7397861	c	g	0.0954	0.0212	-0.01423	0.014274
rs763121	a	g	0.1645	0.0224	-0.00229	0.014303
rs8070740	a	g	-0.1465	0.0243	0.030278	0.015805
rs9039	t	c	0.1248	0.0226	-0.00358	0.015373
rs930036	a	g	-0.1852	0.0207	-0.02011	0.014146
rs9393800	a	g	0.1689	0.0232	-0.00131	0.01552
rs9796	a	t	0.1309	0.0204	-0.01829	0.014033

Table S26. Non-synonymous variants in linkage disequilibrium (r^2>0.8) with the GWAS signals (from HaploReg v2).

SNP from clumped results	Misssense variant	pos (hg19)	LD in EUR		Alleles		Frequency				GENCODE gene	dbSNP functional annotation	Amino acid change	Protein sequence position
			(r^2)	(D')	Ref	Alt	AFR	AMR	ASN	EUR				
Varients at r-sqr > 0.8														
rs2236918	rs735943	chr1:242030151	0.98	0.99	A	G	0.6	0.67	0.78	0.58	*EXO1*	missense	H [His] ⇒ R [Arg]	354
rs4693089	rs1494961	chr4:84374480	1	1	C	T	0.82	0.61	0.65	0.49	*HELQ*	missense	V [Val] ⇒ I [Ile]	506
rs12196873	rs3204953	chr6:111628626	0.91	0.97	C	T	0.01	0.12	0	0.15	*REV3L*	missense	V [Val] ⇒ I [Ile]	2986 / 3064
rs2277339	**rs2277339**	chr12:57146069	1	1	T	G	0.17	0.14	0.24	0.11	*PRIM1*	missense	D [Asp] ⇒ A [Ala]	5
rs3741604	rs4430553	chr12:66698895	0.98	1	T	C	0.61	0.39	0.32	0.53	*HELB*	missense	L [Leu] ⇒ P [Pro]	191
rs1183272	rs1185244	chr12:66725160	0.96	0.99	C	T	0.43	0.57	0.59	0.43	*HELB*	missense	P [Pro] ⇒ L [Leu]	966
rs10852344	rs11544193	chr16:12009304	0.96	0.98	C	A	0.11	0.47	0.15	0.56	*GSPT1*	missense	G [Gly] ⇒ C [Cys]	92
rs8070740	rs12761	chr17:5326145	0.86	0.98	C	G	0.21	0.38	0.81	0.32	*RPAIN*	missense	N [Asn] ⇒ K [Lys]	103
rs1799949	rs1799966	chr17:41223094	0.97	0.99	T	A,C	0.22	0.4	0.33	0.36	*BRCA1*	missense	S [Ser] ⇒ R [Arg	468 / 509 / 510 / 1486 / 1543 / 1566 / 1612 / 1613 / 1634
	rs16942	chr17:41244000	0.99	0.99	T	C	0.22	0.4	0.33	0.36	*BRCA1*	missense	K [Lys] ⇒ R [Arg]	1056 / 1113 / 1136 / 1182 / 1182
	rs16941	chr17:41244435	0.96	0.98	T	C	0.13	0.38	0.33	0.36	*BRCA1*	missense	E [Glu] ⇒ A [Ala]	991 / 968 / 991 / 1037 / 1038
	rs799917	chr17:41244936	0.95	0.99	G	A,T	0.86	0.45	0.33	0.37	*BRCA1*	missense	P [Pro] ⇒ Q [Gln]	744 / 801 / 824 / 870 / 871
	rs8482	chr17:41361960	0.94	0.98	A	G	0.22	0.4	0.33	0.36	*NBR1*	missense	H [His] ⇒ R [Arg]	853 / 923
rs12461110	**rs12461110**	chr19:56320663	1	1	G	A	0.06	0.34	0.24	0.32	*NLRP11*	missense	P [Pro] ⇒ L [Leu]	339 / 438
rs451417	rs236110	chr20:5933108	0.96	0.99	C	A	0.53	0.14	0.17	0.13	*MCM8*	missense	Q [Gln] ⇒ K [Lys]	63
rs16991615	**rs16991615**	chr20:5948227	1	1	G	A	0	0.09	0	0.06	*MCM8*	missense	E [Glu] ⇒ K [Lys]	341
rs1046089	**rs1046089**	chr6:31602967	1	1	G	A	0.53	0.35	0.35	0.36	*PRRC2A*	missense	R [Arg] ⇒ H [His]	1739 / 1740
Variants at r-sqr > 0.6														
rs12142240	rs41293277	chr1:46806550	0.73	0.87	C	T	0.12	0.17	0.02	0.27	*NSUN4*	missense	L [Leu] ⇒ F [Phe]	19
rs4693089	rs12642536	chr4:84383810	0.66	0.99	C	T	0.15	0.5	0.65	0.4	*MRPS18C*	missense	A [Ala] ⇒ T [Thr]	193 / 261 / 299 / 348
rs1054875	rs17803620	chr15:89804043	0.72	0.9	C	T	0.06	0.33	0.3	0.4	*FANCI*	missense	A [Ala] ⇒ V [Val]	86
	rs2283432	chr15:89836228	0.76	0.91	G	C	0.06	0.33	0.31	0.4	*FANCI*	missense	C [Cys] ⇒ S [Ser]	742
rs2941505	rs1877031	chr:17:37814080	0.78	0.9	G	A	0.18	0.53	0.48	0.68	*STARD3*	missense	R [Arg] ⇒ Q [Gln]	117
rs763121	rs12004	chr22:38877461	0.79	1	T	G	0.18	0.22	0.38	0.32	*KDELR3*	missense	V [Val] ⇒ G [Gly]	199

SNPs in bold are those where top associated variant was a non-synonymous variant

Table S27. Functional significance of non-synonymous variants, using SIFT, Polyphen and SWISS-MODEL.

Region	Signal SNP	Alleles	Missense variant	LD (r^2)	LD (D')	Ref	Alt	EUR ALT AF	Gene	dbSNP functional annotation	Aa change (Ref->Alt)	UniProtKB ref	UniProtKB - functional region/metal binding site/ modified residue	UniProtKB - Experimental mutagenesis	SIFT	PolyPhen	Model (SWISS-Model)	Other functional information
1	rs4246511	c/t/0.71	-															
2	rs12142240	t/c/0.68	-															
3	rs1411478	a/g/0.41	-															
4	rs2236918	c/g/0.45	rs735943	0.98	0.99	A	G	0.58	EXO1	missense	Arg (R) -> His (H) at aa 354 (isoform 1) of Human Exonuclease-1	Q9UQ84	none specified	none specified	tolerated	benign	Outside modelled region of 3qea.1.A. Models based on 4q0z poor quality (QMEAN< -8)	none specified
5	rs704795	a/g/0.4	-															
6	rs1800932	a/g/0.81	-															
7	rs930036	a/g/0.38	-															
8	rs16858210	g/a/0.75	-															
9	rs4693089	a/g/0.51	rs1494961	1	1	C	T	0.49	HELQ	missense	Val (V) -> Ile(I) at aa 306 (isoform 1) of Helicase POLQ-like	Q8TDG4	none specified	none specified	tolerated	benign	Model based on 4kit.1.A poor quality (QMEAN4 = -10.34).	none specified
10	rs6856693	a/g/0.58	-															
11	rs427394	g/a/0.41	-															
12	rs11738223	a/g/0.68	-															
13a	rs2241584	a/g/0.38	-															
13b	rs365132	g/t/0.51	-															
14a	rs6899676	a/g/0.8	-															
14b	rs9393800	g/a/0.27	-															
15a	rs2230365	c/t/0.84	-															
15b	rs707938	g/a/0.32	-															
16	rs12196873	a/c/0.85	rs3204953	0.91	0.97	C	T	0.15	REV3L	missense	Val (V) -> Ile(I) at aa 3064 (isoform a) and aa 2986 (isoform b) of DNA polymerase zeta catalytic subunit	O60673	Near near a Cys-A type Zn finger at aa 3042–3057	none specified	deleterious	possibly damaging	Not modelled - no suitable templates.	Variant is in a region of the DNA polymerase zeta catalytic subunit that interacts with p50, both part of the DNA polymerase zeta four subunit complex (Lee at al. PNAS 2014. PMID: 24449906). (Gomez-Llorente et al. Cell Rep 2013. PMID: 24120860). (Baranovskiy AG. J Biol Chem 2012 PMID: 22465957)
17	rs2720044	a/c/0.84	-															
18	rs10957156	a/g/0.76	-															
19	rs4879656	a/c/0.37	-															
20	rs10905065	a/g/0.61	-															
21a	rs11031006	g/a/0.85	-															
21b	rs6484478	g/a/0.74	-															
22	rs10734411	a/g/0.47	-															

Region	Signal SNP	Alleles	Missense variant	LD (r^2)	LD (D')	Ref	Alt	EUR ALT AF	Gene	dbSNP functional annotation	Aa change (Ref->Alt)	UniProtKB ref	UniProtKB - functional region/metal binding site/ modified residue	UniProtKB - Experimental mutagenesis	SIFT	PolyPhen	Model (SWISS-Model)	Other functional information
23	rs2277339	g/t/0.1	rs2277339	1	1	T	G	0.11	PRIM1	missense	D (Asp) ->A (Ala) change at aa 5 of DNA primase small subunit (PriS/p48)	P49642	none specified	none specified	deleterious	possibly damaging	Template 4bpu.1.A (crystal structure of human primase in heterodimeric form) covers amino acids 4-408 with 99.5% sequence identity. Good models (see Supplementary Figure 4) of wt sequence (QMEAN4=-1.06) and variant (QMEAN4=-1.15).	The variant aa results in a loss of negative charge on the surface. Effect of this is not clear – not in the active site or a characterised region for protein-protein interactions. (Kilkenny ML et al. PNAS 2013 PMID: 24043831).
24a	rs3741604	t/c/0.52	rs4430553	0.98	1	T	C	0.53	HELB	missense	L (Leu) -> P (Pro) at aa 191 of DNA helicase B (hDHB or HDHB)	Q8NG08	none specified	none specified	tolerated	benign	Not modelled - no suitable templates.	Located in the N-terminal domain (Guler GD et al. J Biol Chem. 2012. PMID: 22194613)
24b	rs1183272	c/t/0.45	rs1185244	0.96	0.99	C	T	0.43	HELB	missense	P (Pro) -> L (Leu) at aa 966 of DNA helicase B (hDHB or HDHB)	Q8NG08	Next to a predicted phosphoserine residue at aa 907	none specified	tolerated	benign	Not modelled - no suitable templates.	Located in the phosphorylation regulated subcellular localization domain (Guler GD et al. J Biol Chem. 2012. PMID: 22194613)
24c	rs7397861	g/c/0.64	-															
Exome	rs75770066	g/a/0.034	rs75770066	n/a	n/a	n/a	n/a	n/a	HELB	missense	D (Asp) -> G (Gly) aa 506 of DNA helicase B (hDHB or HDHB)	Q8NG08	none specified	D -> A at aa 506, aa 499 and aa 510 at same time leads to loss of RPA1-binding	deleterious	probably damaging	Model based on 3e1s.1.A poor quality (QMEAN4=-18.2, 20.9% sequence identity)	HDHB interacts with RPA-1 via conserved peptide 493-517. Concurrent mutation of three acidic aa in the helicase domain to non-polar residues results in reduced RPA binding (Guler G.D. et al. J Biol Chem 2012). (See Figure 2)
Exome	rs148126992	c/g/0.025	rs148126992	n/a	n/a	n/a	n/a	n/a	HELB	missense	E (Glu) -> D(Asp) aa 522 of DNA helicase B (hDHB or HDHB)	Q8NG08	none specified	none specified	tolerated	benign	Model based on 3e1s.1.A poor quality (QMEAN4=-18.2, 20.9% sequence identity)	Located near the RPA binding domain (Guler G.D. et al. J Biol Chem 2012).(See Figure 2)
25	rs551087	g/a/0.29	-															
26	rs1727326	c/g/0.15	-															
27	rs12824058	g/a/0.43	-															
28	rs4886238	g/a/0.66	-															
29	rs1713460	g/a/0.3	-															
30	rs9796	t/a/0.46	-															
31	rs1054875	t/a/0.4	-															
32	rs9039	c/t/0.28	-															
33	rs10852344	t/c/0.59	rs11544193	0.96	0.98	C	A	0.56	GSPT1	missense	G (Gly) -> C (Cys) change at aa 92 of G1 to S phase transition 1 protein, also known as Eukaryotic peptide chain release factor GTP-binding subunit (ERF3A)	P15170	none specified	none specified	deleterious	possibly damaging	Not modelled - no suitable templates.	Variant aa is near the PAM2 sequences which are required for function, which are required for binding poly(A) binding protein (PABP, down regulates gene expression via mRNA decay) . (Kong C et al. Mol Cell. 2004 PMID: 15099522) (Osawa M et al. RNA. 2012 PMID: 23019593) (Hashimoto Y. Biochem Biophys Res Commun. 2014. PMID: 24569073)
34	rs12599106	a/t/0.51	-															

Region	Signal SNP	Alleles	Missense variant	LD (r^2)	LD (D')	Ref	Alt	EUR ALT AF	Gene	dbSNP functional annotation	Aa change (Ref->Alt)	UniProtKB ref	UniProtKB - functional region/metal binding site/ modified residue	UniProtKB - Experimental mutagenesis	SIFT	PolyPhen	Model (SWISS-Model)	Other functional information
35	rs8070740	a/g/0.76	rs12761	0.86	0.98	C	G	0.32	RPAIN	missense	N (Asn) -> K (Lys) at position 103 in RPAIN (RPA-interacting protein) isoform b	Q86UA6	Lys at aa 103 can form glycyl lysine isopeptide with SUMO in isoform b.	none specified	tolerated	benign	Not modelled - no suitable templates.	RPAIN isoform b transports replication protein A (RPA) into the promyelocytic leukemia (PML) nuclear body (a subnuclear structure) on UV damage to cells. Asn-103 is associated with decreased sumoylation at Arg-114, Arg-121, Arg-142. Sumoylation is required for localisation to the PML nuclear body. (Park J et al. Mol Cell Biol. 2005. PMID: 16135809).
36	rs2941505	a/g/0.32	-															
37	rs1799949	g/a/0.68	rs1799966	0.97	0.99	T	A,C	0.36	BRCA1	missense	T -> C. S (Ser) -> G (Gly) at aa 1613 in isoform 1 and aa 1566 in isoform 3 (both widely expressed) of breast cancer type 1 susceptibility protein	P38398	none specified	none specified	tolerated	benign	Not modelled - no suitable templates.	Located in central disordered region of BRCA1 thought to act as scaffold for protein and DNA binding (Mark WY. J Mol Biol. 2005 PMID: 15571721).
			rs1799966	0.97	0.99	T	A,C	0.36	BRCA1	missense	T -> A. S (Ser) -> C (Cys) at aa 1613 in isoform 1 and aa 1566 in isoform 3 (both widely expressed) of breast cancer type 1 susceptibility protein	P38398	none specified	none specified	deleterious	possibly damaging	Not modelled - no suitable templates.	Located in central disordered region of BRCA1 thought to act as scaffold for protein and DNA binding (Mark WY. J Mol Biol. 2005 PMID: 15571721).
			rs16942	0.99	0.99	T	C	0.36	BRCA1	missense	K (Lys) -> R (Arg) at aa 1183 in isoform 1 and aa 1136 in isoform 3 (both widely expressed) of breast cancer type 1 susceptibility protein	P38398	none specified	none specified	tolerated	benign	Not modelled - no suitable templates.	In domain (aa 772-1292) interacting with p53 (Buck M. Cancer Lett. 2008 PMID: 18501503). Located in central disordered region of BRCA1 thought to act as scaffold for protein and DNA binding (Mark WY. J Mol Biol. 2005 PMID: 15571721).
			rs16941	0.96	0.98	T	C	0.36	BRCA1	missense	E (Glu) -> G(Gly) at aa 1038 in isoform 1 and aa 991 in isoform 3 (both widely expressed) of breast cancer type 1 susceptibility protein	P38398	none specified	none specified	tolerated	benign	Not modelled - no suitable templates.	In domain (aa 772-1292) interacting with p53 (Buck M. Cancer Lett. 2008 PMID: 18501503). Located in central disordered region of BRCA1 thought to act as scaffold for protein and DNA binding. In a suggested DNA binding domain. (Mark WY. J Mol Biol. 2005 PMID: 15571721).
			rs799917	0.95	0.99	G	A,T	0.37	BRCA1	missense	G -> T. P (Pro) -> Q (Gln) at aa 871 in isoform 1 and aa 824 in isoform 3 (both widely expressed) of breast cancer type 1 susceptibility protein	P38398	none specified	none specified	deleterious	benign	Not modelled - no suitable templates.	In domain (aa 772-1292) interacting with p53 (Buck M. Cancer Lett. 2008 PMID: 18501503). Located in central disordered region of BRCA1 thought to act as scaffold for protein and DNA binding. (Mark WY. J Mol Biol. 2005 PMID: 15571721).

Region	Signal SNP	Alleles	Missense variant	LD (r^2)	LD (D')	Ref	Alt	EUR ALT AF	Gene	dbSNP functional annotation	Aa change (Ref->Alt)	UniProtKB ref	UniProtKB - functional region/metal binding site/ modified residue	UniProtKB - Experimental mutagenesis	SIFT	PolyPhen	Model (SWISS-Model)	Other functional information
			rs799917	0.95	0.99	G	A,T	0.37	BRCA1	missense	G -> A. Pro(P) -> L (Leu) at aa 871 in isoform 1 and aa 824 in isoform 3 (both widely expressed) of breast cancer type 1 susceptibility protein	P38398	none specified	none specified	tolerated	benign	Not modelled - no suitable templates.	In domain (aa 772-1292) interacting with p53 (Buck M. Cancer Lett. 2008 PMID: 18501503). Located in central disordered region of BRCA1 thought to act as scaffold for protein and DNA binding. (Mark WY. J Mol Biol. 2005 PMID: 15571721).
			rs8482	0.94	0.98	A	G	0.36	NBR1	missense	His (H) -> Arg (R) change at aa 923 in next to BRCA1 gene 1 protein isoform a	Q14596	Variant aa is in sequence changed in isoform 2	none specified	tolerated	possibly damaging	Template 2mgw.1.A covers the C terminal end of the protein (position 913-959) and includes the variant aa. The template is an NMR structure from Homo sapiens NBR1 (100% sequence identity within the region covered) (Walinda E et al. J Biol Chem. 2014 PMID: 24692539). Good model (QMEAN=-0.63) produced (see Supplementary Figure 4).	In ubiquitin-associated (UBA) domain and dimerisation interface (Walinda E et al. J Biol Chem. 2014 PMID: 24692539)
38	rs349306	g/a/0.13	-															
39	rs7259376	a/g/0.46	-															
40a	rs11668344	g/a/0.36	-															
40b	rs2547274	g/c/0.91	-															
40c	rs12461110	a/g/0.35	rs12461110	1	1	G	A	0.32	NLRP11	missense	Pro (P) -> Leu (L) at aa 438 in isoform 1 of NACHT, LRR and PYD domains-containing protein 11	P59045	Variant aa is in NACHT domain of the protein (147-470 aa), an NTPase with seven conserved motifs.	none specified	tolerated	possibly damaging	Models based on 4kxf.6.A. A poor quality (sequence identity 17.04%; QMEAN4 -12.09 for wt; QMEAN4 -10.77 for rs12461110).	none specified
41a	rs451417	a/c/0.12	rs236110	0.96	0.99	C	A	0.13	MCM8	missense	Gln (Q) -> Lys (K) at aa 63 in MCM8 isoform 1.	Q9UJA3	none specified	none specified	tolerated	benign	Not modelled - no suitable templates.	none specified
41b	rs16991615	g/a/0.93	rs16991615	1	1	G	A	0.06	MCM8	missense	Glu (E) -> Lys (K) at aa 341 in MCM8 isoform 1	Q9UJA3	none specified	none specified	tolerated	benign	Model based on 4fdg1A poor quality (QMEAN4=-9.09 for the wt; QMEAN4=-11.06 for rs16991615).	none specified
42a	rs2236553	c/t/0.24	-															
42b	rs13040088	g/a/0.21	-															
43	rs5762534	t/c/0.84	-															
44	rs763121	g/a/0.36	-															
Exome	rs140267842	a/g/0.009	rs140267842	n/a	n/a	n/a	n/a	n/a	SLCO4A1	missense	Val(V) -> Ile(I) at aa263 of isoform 1 of solute carrier organic anion transporter family member 4A1	Q96BD0	Variant aa is in cytoplasmic domain	none specified	tolerated	possibly damaging	Model based on 1pw4.1.A poor quality (QMEAN4=-10.7)	

HaploReg v2 was used to identify proxy SNPs resulting in missense mutations in LD>0.8 with the
UniprotKB was used to retrieve information about each of the proteins.
The FASTA sequence for each protein (from dbSNP or UniprotKB) was used to search for a template
SIFT and PolyPhen scores are from ENSEMBL.

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Study acknowledgments

1) GWAS

Study name / acronym	Full study name	Acknowledgments and sources of funding
AGES	Age, Gene/ Environment Susceptibility Study	The researchers are indebted to the participants for their willingness to participate in the study. This study has been funded by NIH contract N01-AG-1-2100, the NIA Intramural Research Program, Hjartavernd (the Icelandic Heart Association), and the Althingi (the Icelandic Parliament). The study is approved by the Icelandic National Bioethics Committee, VSN: 00-063.
ARIC	Atherosclerosis Risk in Communities	The Atherosclerosis Risk in Communities Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C), R01HL087641, R01HL59367 and R01HL086694; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health contract HHSN268200625226C. The authors thank the staff and participants of the ARIC study for their important contributions. Infrastructure was partly supported by Grant Number UL1RR025005, a component of the National Institutes of Health and NIH Roadmap for Medical Research. Funding support for "Building on GWAS for NHLBI-diseases: the U.S. CHARGE consortium" was provided by the NIH through the American Recovery and Reinvestment Act of 2009 (ARRA) (5RC2HL102419).
CARL	Cancer Aids Registries Linkage	We are very grateful to the municipal administrators for their collaboration on the project and for logistic support. We would like to thank all participants to this study. We thank Anna Morgan and Angela D'Eustacchio for technical support.
CHS	Cardiovascular Health Study	Cardiovascular Health Study: This CHS research was supported by NHLBI contracts HHSN268201200036C, HHSN268200800007C, HHSN268200960009C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086; and NHLBI grants HL080295, HL087652, HL105756 with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided through AG023629 from the National Institute on Aging (NIA). A full list of CHS investigators and institutions can be found at http://chs-nhlbi.org/. The provision of genotyping data was supported in part by the National Center for Advancing Translational Sciences, CTSI grant UL1TR000124, and the National Institute of Diabetes and Digestive and Kidney Disease Diabetes Research Center (DRC) grant DK063491 to the Southern California Diabetes Endocrinology Research Center.
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COGs	Breast Cancer Association Consortium	This study would not have been possible without the contributions of the following: Per Hall (COGS); Douglas F. Easton, Paul Pharoah, Kyriaki Michailidou, Manjeet K. Bolla, Qin Wang (BCAC), Andrew Berchuck (OCAC), Rosalind A. Eeles, Douglas F. Easton, Ali Amin Al Olama, Zsofia Kote-Jarai, Sara Benlloch (PRACTICAL), Georgia Chenevix-Trench, Antonis Antoniou, Lesley McGuffog, Fergus Couch and Ken Offit (CIMBA), Joe Dennis, Alison M. Dunning, Andrew Lee, and Ed Dicks, Craig Luccarini and the staff of the Centre for Genetic Epidemiology Laboratory, Javier Benitez, Anna Gonzalez-Neira and the staff of the CNIO genotyping unit, Jacques Simard and Daniel C. Tessier, Francois Bacot, Daniel Vincent, Sylvie LaBoissière and Frederic Robidoux and the staff of the McGill University and Génome Québec Innovation Centre, Stig E. Bojesen, Sune F. Nielsen, Borge G. Nordestgaard, and the staff of the Copenhagen DNA laboratory, and Julie M. Cunningham, Sharon A. Windebank, Christopher A. Hilker, Jeffrey Meyer and the staff of Mayo Clinic Genotyping Core Facility.

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Colaus	Etude Cohorte Lausannoise	
CROATIA-Korcula	CROATIA-Korcula	We would like to acknowledge the contributions of the recruitment team in Korcula, the administrative teams in Croatia and Edinburgh and the people of Korcula. The SNP genotyping for the KORCULA cohort was performed in Helmholtz Zentrum München, Neuherberg, Germany. xome array genotyping was performed at the Wellcome Trust Clinical Research Facility Genetics Core at Western General Hospital, Edinburgh, UK. Christian Gieger is supported by Russian Foundation for Basic Research (RFBR)-Helmholtz research group program.

CROATIA-Split	CROATIA-Split	We would like to acknowledge the contributions of the recruitment team from the Croatian Centre for Global Health, University of Split, the administrative teams in Croatia and Edinburgh and the people of Split. The SNP genotyping for the CROATIA_Split cohort was performed by AROS Applied Biotechnology, Aarhus, Denmark. Medical Research Council UK and the Ministry of Science, Education and Sport in the Republic of Croatia (number 108-1080315-0302).
CROATIA-Vis	CROATIA-Vis	We would like to acknowledge the staff of several institutions in Croatia that supported the field work, including but not limited to The University of Split and Zagreb Medical Schools, Institute for Anthropological Research in Zagreb and Croatian Institute for Public Health. The SNP genotyping for the CROATIA_Vis cohort was performed in the core genotyping laboratory of the Wellcome Trust Clinical Research Facility at the Western General Hospital, Edinburgh, Scotland Medical Research Council UK and the Ministry of Science, Education and Sport in the Republic of Croatia (number 108-1080315-0302).
deCODE		
EGCUT (370k)	Estonian Genome Center, University of Tartu	EGCUT work was supported by the Targeted Financing from the Estonian Ministry of Science and Education [SF0180142s08]; the US National Institute of Health [R01DK075787]; the Development Fund of the University of Tartu (grant SP1GVARENG); the European Regional Development Fund to the Centre of Excellence in Genomics (EXCEGEN; grant 3.2.0304.11-0312); and through FP7 grant 313010.
EGCUT OmniX	Estonian Genome Center, University of Tartu	EGCUT work was supported by the Targeted Financing from the Estonian Ministry of Science and Education [SF0180142s08]; the US National Institute of Health [R01DK075787]; the Development Fund of the University of Tartu (grant SP1GVARENG); the European Regional Development Fund to the Centre of Excellence in Genomics (EXCEGEN; grant 3.2.0304.11-0312); and through FP7 grant 313010.
ERF	Erasmus Rucphen Family study	We are grateful to all general practitioners for their contributions, to Petra Veraart for her help in genealogy, JeannetteVergeer for the supervision of the laboratory work and Peter Snijders for his help in data collection. The ERF study as a part of EUROSPAN (European Special Populations Research Network) was supported by European Commission FP6 STRP grant number 018947 (LSHG-CT-2006-01947) and also received funding from the European Community's Seventh Framework Programme (FP7/2007-2013)/grant agreement HEALTH-F4-2007-201413 by the European Commission under the programme "Quality of Life and Management of the Living Resources" of 5th Framework Programme (no. QLG2-CT-2002-01254). High-throughput analysis of the ERF data was supported by joint grant from Netherlands Organization for Scientific Research and the Russian Foundation for Basic Research (NWO-RFBR 047.017.043). Exome sequencing analysis in ERF was supported by the ZonMw grant (project 91111025). Najaf Amin is supported by the Hersenstichting Nederland (project number F2013(1)-28).
FHS	Framingham Heart Study	The authors thank the Framingham Heart Study participants and staff The Framingham Heart Study phenotype-genotype analyses were supported by the National Institute of Aging (Genetics of Reproductive Life Period and Health Outcomes, R21AG032598; JMM, KL and R01AG29451 JMM, KL). The Framingham Heart Study of the National Heart Lung and Blood Institute of the National Institutes of Health and Boston University School of Medicine was supported by the National Heart, Lung and Blood Institute's Framingham Heart Study Contract No. N01-HC-25195 and its contract with Affymetrix, Inc for genotyping services (Contract No. N02-HL-6-4278). Analyses reflect intellectual input and resource development from the Framingham Heart Study investigators participating in the SNP Health Association Resource (SHARe) project. A portion of this research was conducted using the Linux Cluster for Genetic Analysis (LinGA-II) funded by the Robert Dawson Evans Endowment of the Department of Medicine at Boston University School of Medicine and Boston Medical Center. Genotyping, quality control and calling of the Illumina HumanExome BeadChip in the Framingham Heart Study was supported by funding from the National Heart, Lung and Blood Institute Division of Intramural Research (Daniel Levy and Christopher J. O'Donnell, Principal Investigators).

Generation Scotland	Generation Scotland: Scottish Family Health Study	We would like to acknowledge the contributions of the families who took part in the Generation Scotland: Scottish Family Health Study, the general practitioners and Scottish School of Primary Care for their help in recruiting them, and the whole Generation Scotland team, which includes academic researchers, IT staff, laboratory technicians, statisticians and research managers. Genotyping was performed at the Wellcome Trust Clinical Research Facility Genetics Core at Western General Hospital, Edinburgh, UK. Scottish Executive Health Department, Chief Scientist Office, grant number CZD/16/6. Exome array genotyping for GS:SFHS was funded by the Medical Research Council UK
GENOA	Genetic Epidemiology Network of Arteriopathy	Support for the Genetic Epidemiology Network of Arteriopathy (GENOA) was provided by the National Heart, Lung and Blood Institute of the National Institutes of Health (HL054464, HL054457, HL054481, and HL087660). Genotyping was performed at the Mayo Clinic (Stephen Turner, Mariza de Andrade, Julie Cunningham) and was made possible by the University of Texas Health Sciences Center (Eric Boerwinkle, Megan Grove-Gaona). We would also like to thank the families that participated in the GENOA study. HL054464, HL054457, HL054481, and HL087660
HAPI Heart Study	Heredity and Phenotype Intervention (HAPI) Heart Study	U01-HL72515, U01-HL84756, R01-088119, P30-DK072488, K01-HL116770
HealthABC	The Health, Aging, and Body Composition Study	The Health ABC Study was supported by NIA contracts N01AG62101, N01AG62103, and N01AG62106 and, in part, by the NIA Intramural Research Program. The genome-wide association study was funded by NIA grant 1R01AG032098-01A1 to Wake Forest University Health Sciences and genotyping services were provided by the Center for Inherited Disease Research (CIDR). CIDR is fully funded through a federal contract from the National Institutes of Health to The Johns Hopkins University, contract number HHSN268200782096C. This study utilized the high-performance computational capabilities of the Biowulf Linux cluster at the National Institutes of Health, Bethesda, Md. (http://biowulf.nih.gov). see acknowledgement statement
HRS	Health and Retirement Study	HRS is supported by the National Institute on Aging (NIA U01AG009740). The genotyping was funded separately by the National Institute on Aging (RC2 AG036495, RC4 AG039029). Our genotyping was conducted by the NIH Center for Inherited Disease Research (CIDR) at Johns Hopkins University. Genotyping quality control and final preparation of the data were performed by the Genetics Coordinating Center at the University of Washington. U01 AG009740, RC2 AG036495, RC4 AG039029
InChianti	Invecchiare in Chianti	The InCHIANTI study baseline (1998-2000) was supported as a “targeted project” (ICS110.1/RF97.71) by the Italian Ministry of Health and in part by the U.S. National Institute on Aging (Contracts: 263 MD 9164 and 263 MD 821336); the InCHIANTI Follow-up 1 (2001-2003) was funded by the U.S. National Institute on Aging (Contracts: N.1-AG-1-1 and N.1-AG-1-2111); the InCHIANTI Follow-ups 2 and 3 studies (2004-2010) were financed by the U.S. National Institute on Aging (Contract: N01-AG-5-0002);supported in part by the Intramural research program of the National Institute on Aging, National Institutes of Health, Baltimore, Maryland.
INGI-FVG	Genetic Park of Friuli Venezia Giulia Project	We are very grateful to the municipal administrators for their collaboration on the project and for logistic support. We would like to thank all participants to this study. We thank Anna Morgan and Angela D’Eustacchio for technical support. Fondo Trieste (2008) and Regione FVG (L.26.2008)
INGI-VB	Val Borbera Isolated Population Project	We thank the inhabitants of the VB that made this study possible, the local administrations, the Tortona and Genova archdiocese and the ASL-22, Novi Ligure (Al) for support. We also thank Clara Camaschella for data collection supervision and organization of the clinical data collection, Fiammetta Viganò for technical help, Massimiliano Cocca for building the analysis platform. The research was supported by funds from Compagnia di San Paolo, Torino, Italy; Fondazione Cariplo, Italy and Ministry of Health, Ricerca Finalizzata 2008 and CCM 2010, PRIN 2009 and Telethon, Italy to DT.

InterAct cohort/ InterAct cases	European Prospective Investigation into Cancer & Nutrition - InterAct	We thank all EPIC participants and staff for their contribution to the study. We thank staff from the Technical, Field Epidemiology and Data Functional Group Teams of the MRC Epidemiology Unit in Cambridge, UK, for carrying out sample preparation, DNA provision and quality control, genotyping and data-handling work. The EPIC-InterAct study received funding from the European Union (Integrated Project LSHM-CT-2006-037197 in the Framework Programme 6 of the European Community).
KORA F3/ KORA F4	Cooperative Health Research in the Region of Augsburg (follow-up 3) / (follow-up 4)	We thank all the study participants, all members of staff of the Institutes of Epidemiology and the field staff in Augsburg who planned and conducted the study. The KORA study group consists of A. Peters (speaker), R. Holle, K. Strauch, J. Heinrich, R. Leidl, C. Meisinger, and their co-workers, who are responsible for the design and conduct of the KORA studies. The KORA research platform (KORA, Cooperative Health Research in the Region of Augsburg) was initiated and financed by the Helmholtz Zentrum München - German Research Center for Environmental Health, which is funded by the German Federal Ministry of Education and Research and by the State of Bavaria. Furthermore, KORA research was supported within the Munich Center of Health Sciences (MC Health), Ludwig-Maximilians-Universität, as part of LMUinnovativ. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Elisabeth Altmaier - European Union's Seventh Framework Programme (FP7-Health-F5-2012) under Grant agreement No 305280 (MIMOmics). Christian Gieger is supported by Russian Foundation for Basic Research (RFBR)-Helmholtz research group program.
Lifelines	The LifeLInes Cohort Study and Biobank	LifeLines (LifeLines) - We thank Behrooz Z. Alizadeh, Annemieke Boesjes, Marcel Bruinenberg, Noortje Festen, Pim van der Harst, Ilja Nolte, Lude Franke, Mitra Valimohammadi for their help in creating the GWAS database, and Rob Bieringa, Joost Keers, René Oostergo, Rosalie Visser, Judith Vonk for their work related to data-collection and validation. The authors are grateful to the study participants, the staff from the LifeLines Cohort Study and Medical Biobank Northern Netherlands, and the participating general practitioners and pharmacists. Researchers interested in using the LifeLines data must obtain approval for a specific analysis plan from the scientific board of LifeLines to obtain access to the data. Researchers using the data are required to follow the terms of a signed agreement containing a number of clauses designed to ensure protection of privacy and compliance with relevant laws. For further information, contact Harold Snieder (h.snieder@umcg.nl). Elisabeth Altmaier - European Union's Seventh Framework Programme (FP7-Health-F5-2012) under Grant agreement No 305280 (MIMOmics).
MESA	Multi-Ethnic Study of Atherosclerosis	MESA and the MESA SHARe project are conducted and supported by the National Heart, Lung, and Blood Institute (NHLBI) in collaboration with MESA investigators. Support for MESA is provided by contracts N01-HC-95159, N01-HC-95160, N01-HC-95161, N01-HC-95162, N01-HC-95163, N01-HC-95164, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168, N01-HC-95169, UL1-TR-001079, and UL1-TR-000040. MESA Family is conducted and supported by the National Heart, Lung, and Blood Institute (NHLBI) in collaboration with MESA investigators. Genotyping and analysis support was provided by NHLBI grant R01HL071205. Support is provided by grants and contracts R01HL071051, R01HL071205, R01HL071250, R01HL071251, R01HL071258, R01HL071259, by the National Center for Research Resources, Grant UL1RR033176, and the National Center for Advancing Translational Sciences, Grant UL1TR000124. Funding for SHARe genotyping was provided by NHLBI Contract N02-HL-64278. Genotyping was performed at Affymetrix (Santa Clara, California, USA) and the Broad Institute of Harvard and MIT (Boston, Massachusetts, USA) using the Affymetrix Genome-Wide Human SNP Array 6.0.

NHS Illumina Chip / NHS Omni Chip / NHS Affy Chip	Nurses' Health Study	Nurses' Health Study (NHS_BRCA, NHS_T2D, NHS_CHD, NHS_KS, NHS_GA, NHS_CC, NHS_EC, NHS_GO, NHS_MD, NHS2_BRCA, and NHS2_KS). We would like to thank the participants and staff of the NHS and NHSII for their valuable contributions as well as the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY. The authors assume full responsibility for analyses and interpretation of these data. The NHS GWAS were supported by grants from the National Institutes of Health [NCI (CA40356, CA087969, CA055075, CA98233, U01 CA137088, R01 CA059045, R01 CA137178, R01 CA082838, R01 CA131332), NIDDK (DK058845, DK070756), NHGRI (HG004399, HG004728), NHLBI (HL35464), NIAMS (R01 AR056291)].
NTR	Netherlands Twin Register	We like to acknowledge and thank families who take part in the Netherlands Twin Register and the NTR team, which includes academic researchers, IT staff, laboratory technicians, statisticians and research managers. Support for the Netherlands Twin Register studies and research was obtained from the Netherlands Organization for Scientific Research (NWO) and The Netherlands Organisation for Health Research and Development (ZonMW) grants, 904-61-193,480-04-004,400-05-717, Addiction-31160008, 911-09-032, Spinozapremie 56-464-14192, Biobanking and Biomolecular Resources Research Infrastructure (BBMRI –NL, 184.021.007); the European Research Council (ERC-230374); Rutgers University Cell and DNA Repository (NIMH U24 MH068457-06), the Avera Institute, Sioux Falls, South Dakota (USA) and the National Institutes of Health (NIH R01 HD042157-01A1). Part of the genotyping was funded by the Genetic Association Information Network (GAIN) of the Foundation for the National Institutes of Health and Grand Opportunity grants 1RC2 MH089951). We acknowledge support from VU University's Institute for Health and Care Research (EMGO+), the Neuroscience Campus Amsterdam (NCA) and the faculty of Psychology and Education of VU University.
ORCADES	Orkney Complex Disease Study	We would like to acknowledge the invaluable contributions of the research nurses in Orkney, the administrative team in Edinburgh and the people of Orkney. ORCADES was supported by the Chief Scientist Office of the Scottish Government, the Royal Society, the MRC Human Genetics Unit, Arthritis Research UK and the European Union framework program 6 EUROSPAN project (contract no. LSHG-CT-2006-018947).
QIMR	QIMR Berghofer	IMR: We thank the twins and their families for their participation. We also thank Enda Byrne, Anjali Henders, Dixie Statham, Ann Eldridge, Marlene Grace, Kerrie McAloney, and Lisa Bowdler. A portion of the genotyping on which this study was based (Illumina 370K scans on 4300 individuals) was carried out at the Center for Inherited Disease Research, Baltimore (CIDR), through an access award to our late colleague Dr. Richard Todd (Psychiatry, Washington University School of Medicine, St Louis). Funding was provided by the Australian National Health and Medical Research Council (241944, 339462, 89927, 389875, 389891, 389892, 389938, 442915, 442981, 496739, 552485, 552498), the Australian Research Council (A7960034, A79906588, A79801419, DP0770096, DP0212016, DP0343921), the FP-5 GenomEUtwin Project (QLG2-CT-2002-01254), and the U.S. National Institutes of Health (NIH grants AA07535, AA10248, AA13320, AA13321, AA13326, AA14041, MH66206). G.W.M. is supported by the National Health and Medical Research Council (NHMRC) Fellowship Scheme. Statistical analyses were carried out on the Genetic Cluster Computer, which is financially supported by the Netherlands Scientific Organization (NWO 480-05-003).

RSI / RSII / RSIII	Rotterdam Study I, II, III	The generation and management of the Illumina exome chip v1.0 array data for the Rotterdam Study (RS-I) was executed by the Human Genotyping Facility of the Genetic Laboratory of the Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands. The Exome chip array data set was funded by the Genetic Laboratory of the Department of Internal Medicine, Erasmus MC, from the Netherlands Genomics Initiative (NGI)/Netherlands Organisation for Scientific Research (NWO)-sponsored Netherlands Consortium for Healthy Aging (NCHA; project nr. 050-060-810); the Netherlands Organization for Scientific Research (NWO; project number 184021007) and by the Rainbow Project (RP10; Neterlands Exome Chip Project) of the Biobanking and Biomolecular Research Infrastructure Netherlands (BBMRI-NL; www.bbmri.nl). We thank Ms. Mila Jhamai, Ms. Sarah Higgins, and Mr. Marijn Verkerk for their help in creating the exome chip database, and Carolina Medina-Gomez, BSc, Lennard Karsten, BSc, and Dr. Linda Broer for QC and variant calling. Variants were called using the best practice protocol developed by Grove et al. as part of the CHARGE consortium exome chip central calling effort (Grove et al., PLoS One, 2014).The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, Netherlands Organization for the Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam. The authors are grateful to the study participants, the staff from the Rotterdam Study and the participating general practitioners and pharmacists.
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SHIP	Study of Health in Pomerania	SHIP is part of the Community Medicine Research net of the University of Greifswald, Germany, which is funded by the Federal Ministry of Education and Research (grants no. 01ZZ9603, 01ZZ0103, and 01ZZ0403), the Ministry of Cultural Affairs as well as the Social Ministry of the Federal State of Mecklenburg-West Pomerania, and the network ‘Greifswald Approach to Individualized Medicine (GANI_MED)’ funded by the Federal Ministry of Education and Research (grant 03IS2061A). Genome-wide and ExomeChip data have been supported by the Federal Ministry of Education and Research (grants no. 03ZIK012 and 03Z1CN22) and a joint grant from Siemens Healthcare, Erlangen, Germany and the Federal State of Mecklenburg- West Pomerania. The University of Greifswald is a member of the 'Center of Knowledge Interchange' program of the Siemens AG and the Caché Campus program of the InterSystems GmbH. grants no. 01ZZ9603, 01ZZ0103, 01ZZ0403, 03ZIK012, 03Z1CN22 and 03IS2061A
TwinsUKI/ TwinsUKII/ TwinsUKIII		TwinsUK. The study was funded by the Wellcome Trust; European Community’s Seventh Framework Programme (FP7/2007-2013). The study also receives support from the National Institute for Health Research (NIHR) BioResource Clinical Research Facility and Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London. SNP Genotyping was performed by The Wellcome Trust Sanger Institute and National Eye Institute via NIH/CIDR.
WGHS	Women's Genome Health Study	The WGHS is supported by HL043851 and HL080467 from the National Heart, Lung, and Blood Institute and CA047988 from the National Cancer Institute, and the Donald W. Reynolds Foundation, with collaborative scientific support and funding for genotyping provided by Amgen.

2) Exome chip

Study name / acronym	Full study name	Acknowledgments and source of funding
1958BC	1958 National Child Development Study (also known as the 1958 Birth Cohort Study)	This work made use of data and samples generated by the 1958 Birth Cohort (NCDS). Access to these resources was enabled via the 58READIE Project funded by Wellcome Trust and Medical Research Council (grant numbers WT095219MA and G1001799). A full list of the financial, institutional and personal contributions to the development of the 1958 Birth Cohort Biomedical resource is available at http://www2. le. ac. uk/projects/birthcohort. Genotyping was undertaken as part of the Wellcome Trust Case-Control Consortium (WTCCC) under Wellcome Trust award 076113, and a full list of the investigators who contributed to the generation of the data is available at www. wtccc. org. uk. Wellcome Trust grant WT095219MA, Medical Research Council grant G1001799, Wellcome Trust award 076113.
ARIC	Atherosclerosis Risk in Communities HapMap analysis	The Atherosclerosis Risk in Communities Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C), R01HL087641, R01HL59367 and R01HL086694; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health contract HHSN268200625226C. The authors thank the staff and participants of the ARIC study for their important contributions. Infrastructure was partly supported by Grant Number UL1RR025005, a component of the National Institutes of Health and NIH Roadmap for Medical Research. Funding support for “Building on GWAS for NHLBI-diseases: the U.S. CHARGE consortium” was provided by the NIH through the American Recovery and Reinvestment Act of 2009 (ARRA) (5RC2HL102419).
CHS	Cardiovascular Health Study	This CHS research was supported by NHLBI contracts HHSN268201200036C, HHSN268200800007C, HHSN268200960009C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086; and NHLBI grants HL080295, HL087652, HL105756 with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided through AG023629 from the National Institute on Aging (NIA). A full list of CHS investigators and institutions can be found at http://chs-nhlbi.org/. The provision of genotyping data was supported in part by the National Center for Advancing Translational Sciences, CTSI grant UL1TR000124, and the National Institute of Diabetes and Digestive and Kidney Disease Diabetes Research Center (DRC) grant DK063491 to the Southern California Diabetes Endocrinology Research Center.
Fenland		The Fenland Study is funded by the Wellcome Trust and the Medical Research Council, as well as by the Support for Science Funding programme and CamStrad. We are grateful to all the volunteers for their time and help, and to the General Practitioners and practice staff for help with recruitment. We thank the Fenland Study co-ordination team and the Field Epidemiology team of the MRC Epidemiology Unit for recruitment and clinical testing

FHS	Framingham Heart Study	The authors thank the Framingham Heart Study participants and staff The Framingham Heart Study phenotype-genotype analyses were supported by the National Institute of Aging (Genetics of Reproductive Life Period and Health Outcomes, R21AG032598; JMM, KL and R01AG29451 JMM, KL). The Framingham Heart Study of the National Heart Lung and Blood Institute of the National Institutes of Health and Boston University School of Medicine was supported by the National Heart, Lung and Blood Institute's Framingham Heart Study Contract No. N01-HC-25195 and its contract with Affymetrix, Inc for genotyping services (Contract No. N02-HL-6-4278). Analyses reflect intellectual input and resource development from the Framingham Heart Study investigators participating in the SNP Health Association Resource (SHARe) project. A portion of this research was conducted using the Linux Cluster for Genetic Analysis (LinGA-II) funded by the Robert Dawson Evans Endowment of the Department of Medicine at Boston University School of Medicine and Boston Medical Center. Genotyping, quality control and calling of the Illumina HumanExome BeadChip in the Framingham Heart Study was supported by funding from the National Heart, Lung and Blood Institute Division of Intramural Research (Daniel Levy and Christopher J. O'Donnell, Principal Investigators).
INGI-VB	Val Borbera Isolated Population Project	We thank the inhabitants of the VB that made this study possible, the local administrations, the Tortona and Genova archdiocese and the ASL-22, Novi Ligure (Al) for support. We also thank Clara Camaschella for data collection supervision and organization of the clinical data collection, Fiammetta Viganò for technical help, Massimiliano Cocca for building the analysis platform. The research was supported by funds from Compagnia di San Paolo, Torino, Italy; Fondazione Cariplo, Italy and Ministry of Health, Ricerca Finalizzata 2008 and CCM 2010, PRIN 2009 and Telethon, Italy to DT.
InterAct Cases/ InterAct Subcohort	European Prospective Investigation into Cancer & Nutrition - InterAct	We thank all EPIC participants and staff for their contribution to the study. We thank staff from the Technical, Field Epidemiology and Data Functional Group Teams of the MRC Epidemiology Unit in Cambridge, UK, for carrying out sample preparation, DNA provision and quality control, genotyping and data-handling work. The EPIC-InterAct study received funding from the European Union (Integrated Project LSHM-CT-2006-037197 in the Framework Programme 6 of the European Community).
KORA	Cooperative Health Research in the Region of Augsburg (follow-up 4)	We thank all the study participants, all members of staff of the Institutes of Epidemiology and the field staff in Augsburg who planned and conducted the study. The KORA study group consists of A. Peters (speaker), R. Holle, K. Strauch, J. Heinrich, R. Leidl, C. Meisinger, and their co-workers, who are responsible for the design and conduct of the KORA studies. The KORA research platform (KORA, Cooperative Health Research in the Region of Augsburg) was initiated and financed by the Helmholtz Zentrum München - German Research Center for Environmental Health, which is funded by the German Federal Ministry of Education and Research and by the State of Bavaria. Furthermore, KORA research was supported within the Munich Center of Health Sciences (MC Health), Ludwig-Maximilians-Universität, as part of LMUinnovativ. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Elisabeth Altmaier - European Union's Seventh Framework Programme (FP7-Health-F5-2012) under Grant agreement No 305280 (MIMOmics). Christian Gieger is supported by Russian Foundation for Basic Research (RFBR)-Helmholtz research group program.
MESA	Multi-Ethnic Study of Atherosclero sis	MESA and the MESA SHARe project are conducted and supported by the National Heart, Lung, and Blood Institute (NHLBI) in collaboration with MESA investigators. Support for MESA is provided by contracts N01-HC-95159, N01-HC-95160, N01-HC-95161, N01-HC-95162, N01-HC-95163, N01-HC-95164, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168, N01-HC-95169, UL1-TR-001079, and UL1-TR-000040. MESA Family is conducted and supported by the National Heart, Lung, and Blood Institute (NHLBI) in collaboration with MESA investigators. Support is provided by grants and contracts R01HL071051, R01HL071205, R01HL071250, R01HL071251, R01HL071258, R01HL071259, by the National Center for Research Resources, Grant UL1RR033176, and the National Center for Advancing Translational Sciences, Grant UL1TR000124. Funding for SHARe genotyping was provided by NHLBI Contract N02-HL-64278. Genotyping was performed at Affymetrix (Santa Clara, California, USA) and the Broad Institute of Harvard and MIT (Boston, Massachusetts, USA) using the Affymetrix Genome-Wide Human SNP Array 6. 0.

Amish	Old Order Amish Study	U01-HL72515, U01-HL84756, R01-088119, P30-DK072488, K01-HL116770
Cambridge Cancer	The EMBRACE, SEARCH (breast cancer and ovarian cancer) and SIBS studies	Douglas F. Easton is the PI of the study. EMBRACE Collaborating Centres are: Coordinating Centre, Cambridge:Debra Frost, Steve Ellis, Radka Platte, Jo Perkins. North of Scotland Regional Genetics Service, Aberdeen: Zosia Miedzybrodzka, Helen Gregory. Northern Ireland Regional Genetics Service, Belfast: Patrick Morrison, Lisa Jeffers. West Midlands Regional Clinical Genetics Service, Birmingham: Kai-ren Ong, Jonathan Hoffman. South West Regional Genetics Service, Bristol: Alan Donaldson, Margaret James. East Anglian Regional Genetics Service, Cambridge: Joan Paterson, Marc Tischkowitz, Sarah Downing, Amy Taylor. Medical Genetics Services for Wales, Cardiff: Alexandra Murray, Mark T. Rogers, Emma McCann. St James's Hospital, Dublin & National Centre for Medical Genetics, Dublin: M. John Kennedy, David Barton. South East of Scotland Regional Genetics Service, Edinburgh: Mary Porteous, Sarah Drummond. Peninsula Clinical Genetics Service, Exeter: Carole Brewer, Emma Kivuva, Anne Searle, Selina Goodman, Kathryn Hill. West of Scotland Regional Genetics Service, Glasgow: Rosemarie Davidson, Victoria Murday, Nicola Bradshaw, Lesley Snadden, Mark Longmuir, Catherine Watt, Sarah Gibson, Eshika Haque, Ed Tobias, Alexis Duncan. South East Thames Regional Genetics Service, Guy's Hospital London: Louise Izatt, Chris Jacobs, Caroline Langman. North West Thames Regional Genetics Service, Harrow: Huw Dorkins. Leicestershire Clinical Genetics Service, Leicester: Julian Barwell. Yorkshire Regional Genetics Service, Leeds: Julian Adlard, Gemma Serra-Feliu. Cheshire & Merseyside Clinical Genetics Service, Liverpool: Ian Ellis, Claire Foo. Manchester Regional Genetics Service, Manchester: D Gareth Evans, Fiona Lalloo, Jane Taylor. North East Thames Regional Genetics Service, NE Thames, London: Lucy Side, Alison Male, Cheryl Berlin. Nottingham Centre for Medical Genetics, Nottingham: Jacqueline Eason, Rebecca Collier. Northern Clinical Genetics Service, Newcastle: Alex Henderson, Oonagh Claber, Irene Jobson. Oxford Regional Genetics Service, Oxford: Lisa Walker, Diane McLeod, Dorothy Halliday, Sarah Durell, Barbara Stayner. The Institute of Cancer Research and Royal Marsden NHS Foundation Trust: Ros Eeles, Nazneen Rahman, Elizabeth Bancroft, Elizabeth Page, Audrey Ardern-Jones, Kelly Kohut, Jennifer Wiggins, Jenny Pope, Sibel Saya, Natalie Taylor, Zoe Kemp and Angela George. North Trent Clinical Genetics Service, Sheffield: Jackie Cook, Oliver Quarrell, Cathryn Bardsley. South West Thames Regional Genetics Service, London: Shirley Hodgson, Sheila Goff, Glen Brice, Lizzie Winchester, Charlotte Eddy, Vishakha Tripathi, Virginia Attard. Wessex Clinical Genetics Service, Princess Anne Hospital, Southampton: Diana Eccles, Anneke Lucassen, Gillian Crawford, Donna McBride, Sarah Smalley. CRUK ref: C8197/A16565, CRUK ref: C1287/A8459, CRUK ref: A490/A10124 EMBRACE is supported by Cancer Research UK Grants C1287/A10118, C1287/A16563 and C1287/A17523. Genotyping was supported by Cancer Research–UK grant C12292/A11174D. Gareth Evans and Fiona Lalloo are supported by an NIHR grant to the Biomedical Research Centre, Manchester. The Investigators at The Institute of Cancer Research and The Royal Marsden NHS Foundation Trust are supported by an NIHR grant to the Biomedical Research Centre at The Institute of Cancer Research and The Royal Marsden NHS Foundation Trust. Ros Eeles and Elizabeth Bancroft are supported by Cancer Research UK Grant C5047/A8385.
deCODE		

EGCUT	Estonian Genome Center, University of Tartu	EGCUT work was supported by the Targeted Financing from the Estonian Ministry of Science and Education [SF0180142s08]; the US National Institute of Health [R01DK075787]; the Development Fund of the University of Tartu (grant SP1GVARENG); the European Regional Development Fund to the Centre of Excellence in Genomics (EXCEGEN; grant 3.2.0304.11-0312); and through FP7 grant 313010.
Generation Scotland	Generation Scotland: Scottish Family Health Study	We would like to acknowledge the contributions of the families who took part in the Generation Scotland: Scottish Family Health Study, the general practitioners and Scottish School of Primary Care for their help in recruiting them, and the whole Generation Scotland team, which includes academic researchers, IT staff, laboratory technicians, statisticians and research managers. Genotyping was performed at the Wellcome Trust Clinical Research Facility Genetics Core at Western General Hospital, Edinburgh, UK. Scottish Executive Health Department, Chief Scientist Office, grant number CZD/16/6. Exome array genotyping for GS:SFHS was funded by the Medical Research Council UK
Korcula	CROATIA_Korcula	We would like to acknowledge the contributions of the recruitment team in Korcula, the administrative teams in Croatia and Edinburgh and the people of Korcula. The SNP genotyping for the KORCULA cohort was performed in Helmholtz Zentrum München, Neuherberg, Germany. xome array genotyping was performed at the Wellcome Trust Clinical Research Facility Genetics Core at Western General Hospital, Edinburgh, UK. Medical Research Council UK and the Ministry of Science, Education and Sport in the Republic of Croatia (number 108-1080315-0302).
Rotterdam	Rotterdam Study I	The generation and management of the Illumina exome chip v1.0 array data for the Rotterdam Study (RS-I) was executed by the Human Genotyping Facility of the Genetic Laboratory of the Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands. The Exome chip array data set was funded by the Genetic Laboratory of the Department of Internal Medicine, Erasmus MC, from the Netherlands Genomics Initiative (NGI)/Netherlands Organisation for Scientific Research (NWO)-sponsored Netherlands Consortium for Healthy Aging (NCHA; project nr. 050-060-810); the Netherlands Organization for Scientific Research (NWO; project number 184021007) and by the Rainbow Project (RP10; Neterlands Exome Chip Project) of the Biobanking and Biomolecular Research Infrastructure Netherlands (BBMRI-NL; www.bbmri.nl). We thank Ms. Mila Jhamai, Ms. Sarah Higgins, and Mr. Marijn Verkerk for their help in creating the exome chip database, and Carolina Medina-Gomez, BSc, Lennard Karsten, BSc, and Dr. Linda Broer for QC and variant calling. Variants were called using the best practice protocol developed by Grove et al. as part of the CHARGE consortium exome chip central calling effort (Grove et al., PLoS One, 2014). The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, Netherlands Organization for the Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam. The authors are grateful to the study participants, the staff from the Rotterdam Study and the participating general practitioners and pharmacists.
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WGHS	Women's Genome Health Study	The WGHS is supported by HL043851 and HL080467 from the National Heart, Lung, and Blood Institute and CA047988 from the National Cancer Institute, and the Donald W. Reynolds Foundation, with collaborative scientific support and funding for genotyping provided by Amgen.
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3) Individual Study disclosures

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1. Gusev, A., et al., Partitioning heritability of regulatory and cell-type-specific variants across 11 common diseases. Am J Hum Genet, 2014. 95(5): p. 535-52.
2. Bulik-Sullivan, B., et al., An Atlas of Genetic Correlations across Human Diseases and Traits. 2015.

Chapter 7: Discussion

In this thesis I have presented a number of studies that have contributed to our understanding of the genetic and non-genetic factors influencing female reproductive ageing. In this section I discuss how effective these studies have been, how these results have benefited our overall knowledge, common themes within the results and directions for future research.

Events before birth influence age at menopause

In Chapter 2, we describe a study in which we found an association between being part of a multiple birth and earlier menopause, with an effect size as large as that of smoking. Our results demonstrate a link between events around the time of formation of the ovarian follicle pool and reproductive ageing in later life, either as a result of differences in the number of follicles formed or the rate of decline in follicles. Interestingly, an earlier published study found an increased rate of primary ovarian insufficiency (POI) in both monozygotic and dizygotic twins, suggesting that twin's shared environment may contribute to earlier menopause rather than genetic factors[1].

Intrauterine growth restriction does not appear to affect ovary development[2], and we suggest that the connection with multiple births may be mediated though pregnancies in older mothers, since the rate of multiple births increases with mother's age[3]. Although we did not find evidence of a relationship between older mothers and early menopause in their offspring, this may be because we were unable to adjust for mother's socio-economic status, a potential confounder. Genetic abnormalities, hypertension and birth complications are all more common in older mothers, but the genetic variants affecting DNA repair discussed in Chapter 6 may also play a role, and such environmental factors are likely to have different effects depending on the genetic variants present.

Birthweight has shown various relationships in other studies[4-7] and the effect of birthweight was less strong in our study than multiple births, though we were unable to control for gestational age which influences birthweight. The effect of birthweight on age at menopause could be explored using Mendelian Randomisation analysis, by using instrumental variables based on the genetic

variants for birthweight that have been identified[8], however this approach will not be suitable for epidemiological factors not clearly influenced by genetics.

Other non-genetic risk factors affect age at menopause

A wide-range of non-genetic risk factors have been suggested to be associated with age at menopause, and in Chapter 2, consistent with the reported relationships we found that odds of early menopause were increased by smoking[9-15], having decreased levels of education [9,12,13,16] and being nulliparous [10,14]. We also found associations of earlier menopause with earlier age at menarche, thinner body size at age 10 and earlier year of birth, as suggested by previous studies[4,10,17-22]. However, we did not find consistent associations between age at menopause and BMI[10,14], socio-economic status and not eating meat [14,23,24]. Even in this large, well-powered study, controlling for potential confounders is challenging. The Mendelian Randomisation approach mentioned above provides one method for analysis that avoids issues of confounding.

Common genetic control of menstrual cycle and menopause

The results presented in Chapter 3, 4 and 6 demonstrate commonality between the genetics of hormone levels, menstrual cycle length and age at menopause. We found associations of genetic variants near the *FSHB* gene with levels of FSH and LH (Chapter 3)[25], and length of menstrual cycle, endometriosis and fertility (Chapter 4). The *FSHB* locus had previously been identified as associated with menopause age[26], and we replicated this association in the analysis presented in Chapter 6. This locus has also been associated with PCOS in other studies[27,28], though we found no such association in our analysis, probably as we were under-powered (Chapter 4).

Experimental evidence suggests that the causal variant is most likely to be a polymorphism in the promoter of *FSHB*, 211 bp upstream of the transcription start site, which reduces *FSHB* transcription [29,30]. In Chapter 3, it was shown that the *FSHB* polymorphism has the opposite effect on levels of LH and FSH, though we do not yet understand the how this impacts the biology of reproductive traits. While we would intuitively expect control of the menstrual cycle and reproductive ageing to be connected, these results provide genetic evidence for this link.

The study presented in Chapter 4 demonstrates how we are able to study a wide-range of reproductive phenotypes in a large number of women in a population based sample by using data from the UK Biobank[31]. We were able to detect a novel genetic association even though we only had ~10,000 women with menstrual cycle in this first release of genotyped data. However, the finding needs to be replicated and this should be possible with the next batch of genotyped data released by UK Biobank.

In the future, with the release of genetic data for all 270,000 women from UK Biobank we will have improved power to identify other loci with relevance to age at menopause and other reproductive phenotypes. This will also enable us to perform bivariate analysis of age at menopause and other phenotypes, in a similar way to that carried out previously for menarche and menopause [32].

Overlaps in the genetic control of sex hormones

The results of our study in Chapter 3 suggest that overlaps in the genetic basis of sex hormone regulation are more widespread than just at the *FSHB* locus. We identified two significant genome-wide association studies (GWAS) signals for progesterone, one of which was in a region previously reported as associated with DHEAS near *CYP3A7*[33]. There were indications of a more general overlap in the genetic control of DHEAS and progesterone suggesting that genetic variation might affect the levels of the common precursor pregnenolone.

A new signal for free androgen index (FAI) was identified, which was associated with decreased DHEAS and increased testosterone. We also, identified a novel signal for oestradiol and replicated signals for DHEAS and SHBG[33-35]. It will be of interest to explore these overlaps in the context of female reproductive ageing to investigate whether there are other loci that are common to multiple reproductive phenotypes in addition to the *FSHB* locus.

The main limitation of the hormone GWAS described in Chapter 3 was the lack of available replication data for many of the hormones. Biomarkers are being measured in the UK Biobank and this would give us the opportunity to replicate the oestradiol signal and conduct GWAS for testosterone and SHBG that are larger in size than previous studies[34-36]. Although levels of DHEAS and

progesterone will not be measured in UK Biobank, the availability of more wide-ranging reproductive phenotypes will provide opportunities to test the loci associated with these hormones against other reproductive traits. Additionally, Mendelian Randomisation analyses could be carried out to test the effect of sex hormones on age at menopause.

Large genomic studies remain important for identifying genetics of age at menopause

In Chapter 6, I presented a large meta-analysis of GWAS that more than doubled the number of known genetic loci for age at menopause[37]. This study demonstrates the continued value of large genomic studies and how improvements in sample size can greatly benefit the number of genetic signals identified. In this case, doubling the sample size to ~70,000 women more than doubled the number of signals identified, from 17 to 56[26,37-39]. Despite this improvement, these variants only account for 6% of population variation in age at menopause[40-43], though at least half of variation in menopause age is thought to be due to genetics[42].

The meta-analysis in Chapter 6 is also an improvement on previous GWAS in that low frequency coding variants (<1% minor allele frequency (MAF)) were included by analysing exome chip data, whereas the previous meta-analysis included only variants of >1% MAF[26]. Generally, within complex trait genetics, it was anticipated that exome chip analysis would contribute to the identification of rare variants in coding regions with large effects. It had also been suggested that associations at some common signals might arise by tagging undetected rare variants causing 'synthetic associations'[44,45]. While exome chip studies of many complex traits have not identified rare variant signals[46], we identified low frequency signals at *HELB* (3.6% MAF) and a rare signal (0.8% MAF) at *SLCO4A1* with large effect sizes. At *HELB*, there was a 'synthetic association' with the index signal from the HapMap2 imputed GWAS explained fully by the exome chip signals, though there were other independent common signals not explained by the exome chip signals. At *SLCO4A1* the HapMap2 and exome chip signals were non-redundant and there was no 'synthetic association'.

The major omission from all the meta-analyses for age at menopause carried out so far is the X chromosome. In previous analyses, this was not included as the X chromosome had not tended to be imputed by individual studies for various technical reasons[47]. The X chromosome makes up 5% of the human genome and would be expected to be of particular interest given that approximately 15% of genes known to be involved in POI reside on the X chromosome (though this is likely to be an overestimate of the actual number of POI genes on the X chromosome given its preferential inclusion in many studies). It should now be possible to include X chromosome data since there are standard methods for imputation[47] and statistical analysis of the X chromosome for a female-only trait such as menopause is less complicated than for traits including both sexes.

Further improvements will be made by the next *ReproGen* consortium genome-wide analysis that is currently in progress, which is 1000 Genomes Phase 3 imputed and includes X chromosome data. In addition, we will be including the first release of the UK Biobank genotyped data which is UK10K/1000 Genomes imputed and includes variants at allele frequencies <1%. This genome-wide analysis will result in an improvement on the sample size to around 100,000 women and should increase the number of variants included from ~2.4 million to ~11 million. Additionally, the data will include insertions and deletions which were not included in the genome-wide analysis presented in Chapter 6.

Genome-wide analysis for height, the best characterised complex genetic trait, demonstrates how continued improvements in the number of samples benefit the number of genetic variants identified. For height, an improvement in sample size from ~130,000 to ~250,000 individuals resulted in an additional 243 loci being identified (from 180 to 423 loci) explaining an extra 6% of population variation (from 10% to 16%)[48,49]. This suggests that the next *ReproGen* meta-analysis should increase the percentage variance explained by another few percent and result in the identification of many additional loci. The increased level of detail will aid fine-mapping and the identification of causal variants, for example, benefitting our understanding of the relationships between the low frequency and common signals at *HELB* and *SLCO4A1*.

DNA damage response pathways are involved in menopause timing

In Chapter 6, we used the results of the large genome-wide analysis to identify several biological pathways associated with age at menopause. While the previous *ReproGen* meta-analysis in 2012 suggested the involvement of DNA repair and immune function genes[26], our latest analysis demonstrated a wider involvement of the DNA damage response, particularly relating to homologous recombination, and with common pathways linking menopause and breast cancer. Surprisingly, we found that the epidemiologically observed association of increased breast cancer with later menopause appears to be mediated through sex hormone exposure rather than these DNA damage response genes.

While the genes identified were the most likely based on bioinformatics approaches, experimental studies are still required to confirm their involvement and to understand the effects of these genetic variants. Identifying likely causal variants and relevant genes is one of the major challenges of genome-wide analysis. The most significant signal in a region is not necessarily the causal variant, since it may be tagging other genetic variants in the same haplotype block that are not imputed or genotyped[50]. Also, the causal gene is not necessarily the closest gene to the most significant signal, since the variant might lie in an uncharacterised regulatory region for a distant gene[51].

In Chapter 6, we considered a number of criteria to identify likely causal genes. Likely causal genes were: (i) identified by a gene prioritisation or pathway program; (ii) were an expression quantitative trait locus (eQTL) for the signal; (iii) contained a coding variant which was a top signal or was in strong linkage disequilibrium with the top signal; (iv) were selected on the basis of biology; or, (v) were the nearest gene to the signal. We used molecular modelling approaches and literature searches to understand the functional effects of coding variants, however these were not particularly informative. Biological pathway identification using the program MAGENTA was much more successful, highlighting enrichment of signals in DNA damage response, delayed puberty and premature ovarian failure pathways.

There are limitations to the approaches used in Chapter 6. Although the eQTL data that was analysed included a diverse range of tissues and was from over 100 data sets[52,53], current expression data does not include reproductive organs. Gene expression will differ by cell type and will depend on the context and environment of the cell. Therefore, the absence of an association signal with gene expression does not mean that that variant is not associated. The methods used for biological pathway identification are limited by our knowledge of gene interactions, protein functions and cell biology, which can vary hugely depending on context. Therefore, results will still need to be confirmed experimentally even if the *in silico* evidence appears convincing.

Genes are involved in reproductive disorders and normal reproductive ageing

The signals from the large genome-wide analysis in Chapter 6 were also enriched for genes known to be involved in monogenic causes of POI and delayed puberty. The overlap with genes involved in delayed puberty provides evidence of neural control for of both the start and end of reproductive lifespan. These results demonstrate how different types of variation affecting the same gene can result in different phenotypes ranging from normal variation in age at menopause to monogenic reproductive disorders.

The signals from the large genome-wide analysis in Chapter 6 were near to four POI genes (*MCM8*[54], *EIF2B4*[55], *POLG*[56-58], *MSH5*[59]) and a fifth was near a binding partner of FMRP, the product of *FMR1* (*TDRD3*) [60,61-65]. It will be of interest to see whether the ongoing *ReproGen* 1000 Genomes imputed meta-analysis identifies further overlaps between genes for POI and normal variation in age at menopause, which might highlight additional common biological pathways that will help us to understand the biological basis of reproductive ageing.

In contrast, the study presented in Chapter 5, which shows that normal length *FMR1* alleles are not associated with age at menopause, is an example of how a gene can be involved in POI but have no discernible effect on normal variation in age at menopause (though this may be too small to detect). Previous evidence suggested an association between variation in length of normal *FMR1*

alleles with ovarian reserve, which seemed plausible given the well-established association of premutation length repeats with increased risk of POI, though another study found no evidence of this[66-69]. In conjunction with the previous study[69], our population-based study presented in Chapter 5 contradicts the findings of smaller studies in fertility patients and helps to resolve this controversy.

The study presented in Chapter 5 also suggests that further work is required to confirm the associations of the POI genes listed in Appendix 3 before using them to search for enrichment in GWAS results. The mutations identified as causing POI should be evaluated in the context of the evidence for their association and their frequency in resources such as the ExAC database (http://exac.broadinstitute.org). Population studies, such as GWAS, can also be useful for estimating the penetrance of Mendelian mutations. For example, a recent genome-wide exome chip meta-analysis of menarche age identified a rare variant in *TACR3* with a MAF of 0.08% that is associated with later menarche age of 1.25 years per allele and has previously been reported as a pathogenic mutation causing idiopathic hypogonadotropic hypogonadism[70].

Due to publication bias, negative associations between genes and POI may be under-represented. Care needs to be taken with genes suggested as involved with POI on the basis of their association with age at menopause, which due to the challenges of identifying causal genes from GWAS may lead us to the wrong gene. The availability of genome sequencing data in rare diseases, to be made available by projects such as 100,000 Genomes, should benefit the identification of additional POI genes.

Considerations for future genomic studies of menopause age

In the age at menopause GWAS (Chapter 6), gene burden tests of the exome chip data did not identify any additional loci that were not identified from single-variant analyses, probably due to insufficient coverage of rare coding variants. Other future approaches to capturing rare, coding variation that should be considered include meta-analyses of exome sequencing studies, which allow nearly all protein-coding variants in an individual to be identified[71]. Rare variants are under-powered to be detected as a single variant associations, however by analysing the combined effects of rare variants with similar functions in the

same gene using gene-burden tests, we should be able to identify further causal genes[72].

Future large genomic analysis should consider including studies imputed to a greater level of detail than 1000 Genomes Phase 3, e.g. as with the UK Biobank genetic data[73]. Detailed imputation panels are now becoming available, developed from multi-ethnic low-pass sequencing study data (e.g. UK10K, Haplotype Reference Consortium), which allow better imputation of low frequency variation[74]. As well as better capturing coding variation, these more detailed reference panels will allow us to better identify non-coding variation, which might lie in regulatory regions. One estimate is that up to three-quarters of polymorphisms associated with genetic disease occur within *cis*-regulatory regions[75]. One of the challenges will be to establish whether significant signals lie in such regions and to which gene they relate, since such regions can be several megabases distant from genes.

It will be important to conduct GWAS in other ethnicities in addition to the white European women considered in this thesis. Allele frequencies and haplotype structure vary greatly by ethnicity meaning that the genetic variants identified will depend on the population studied and genes discovered in white women may not translate to other ethnicities. Multi-ethnic analysis approaches can be utilised that use these differences to increase statistical power, by highlighting variants, genes and pathways that are common across ethnicities[76]. Differences in haplotype structure at loci common to different ethnicities can be used to help with the fine-mapping of signals[76]. Additionally, future studies should consider analysis of copy number variants (CNVs). It has been estimated that 77% of common CNVs with allele frequencies greater than 5% are tagged by SNPs and would have therefore already been included in GWAS[77]. However, this leaves rarer, untagged CNVs that remain to be analysed.

Summary

The projects in this thesis have identified additional genetic and non-genetic factors that contribute to variation in age at menopause. Work in this thesis has demonstrated how factors impacting age at menopause overlap more generally with reproductive function, and it will be interesting to explore this further in the future. Understanding the factors that affect age at menopause will improve our

understanding of the biological processes leading to menopause. This should facilitate the development of predictive models for age at menopause which would allow women to make informed choices regarding their health. Previously, a model based on four genetic variants predicted age at menopause with a ROC score of 0.6[78]. The inclusion of the 56 genetic signals identified in Chapter 6, along with the early life risk factors characterised in Chapter 2 should improve this model. However, only a small proportion of the variation in age at menopause has been accounted for. Future studies are required to identify further genetic variants, clarify the impact of epidemiological risk factors and to evaluate the impact of age at menopause on health outcomes.

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Appendices

Appendix 1. Health outcomes associated with age at menopause.

Outcome	Effect on outcome	Effect of variable	Analysis type	Study	n	Birth year of cohort	Reference
Aortic aneurism	Earlier menopause → bigger aortic aneurism	Mean age at menopause lower in women with AAA>5 cm vs AAA<5 cm and women with PAD (47.7 vs 49.9vs 49.7 years, P=0.011).	Chi-squared test, t-tests, non-parametric tests.	Karolinska University Hospital, Stockholm, Sweden	140 women with abdominal aortic aneurism and 140 with peripheral arterial disease	1917-1958 (aged 51-92 in 2009)	Villard, Swedenborg et al. Reproductive history in women with abdominal aortic aneurysms. *J Vasc Surg*. **54**, 341-345, 345.e341-342.(2011)
Autoimmune diseases	POI → increased risk of autoimmune disease	For autoimmune diseases HR=1.58 (95% CI 1.04-2.38). Similar when HRT users excluded. Note: 73% of POF cases were natural.	Logistic regression adjusted for age, income, occupation, BMI, waist hip ratio, smoking, nulliparity, age at menarche, type of menopause and HRT use.	Shanghai Women's Health Study, China	1003 cases POI	1926-1960 (40-70 years 1996-2000)	Wu, Cai et al. Impact of premature ovarian failure on mortality and morbidity among Chinese women. *PLoS One*. **9**, e89597.(2014)
Autoimmune, rheumatoid arthritis	Early menopause - > Milder rheumatoid arthritis	Type of RA differs by age at menopause. Severe RA EM=15.8%, normal menopause=34.8%; mild/moderate seropositive RA EM=26.3%, normal menopause=45.5%; mild/moderate seronegative RA EM=57.9%, normal menopause=19.7%	ANOVA, early menopause (<46 years) vs normal menopause by severity of rheumatoid arthritis.	Malmö Diet and Cancer Study linked to rheumatoid arthritis disease register	134	1927-1947 (aged 44-74 in 1991)	Pikwer, Nilsson et al. Early menopause and severity of rheumatoid arthritis in women older than 45 years. *Arthritis Res Ther*. **14**, R190.(2012)
Autoimmune, rheumatoid arthritis	Early menopause → increased rheumatoid arthritis	Menopause ≤45 years, rheumatoid arthritis OR=1.92 (95% CI 1.02-3.64)	Matched case-control. Odds ratio adjusted for smoking, education, and length of breastfeeding.	Health survey linked to rheumatoid arthritis disease registers	Cases of RA from survey of 18,326 women.		Pikwer, Bergstrom et al. Early menopause is an independent predictor of rheumatoid arthritis. *Ann Rheum Dis*. **71**, 378-381.(2012)
Autoimmune, sarcoidosis	Earlier menopause → increased risk of sarcoidosis	Women having a natural or surgical menopause. Compared with menopause <40 years (ref), 40-44 years HR=0.92 (95% CI 0.51-1.67), 45-49 years HR=0.65 (95% 0.34-1.22), 50+ years (95% CI 0.31-1.15), p-trend=0.03	Cox PH adjusted for age, education, geographic region, smoking, BMI, questionnaire cycle.	Black Women Heath Study, USA	56,886	1926-1974 (aged 21-69 in 1995)	Cozier, Berman et al. Reproductive and hormonal factors in relation to incidence of sarcoidosis in US Black women: The Black Women's Health Study. *Am J Epidemiol*. **176**, 635-641.(2012)

Outcome	Effect on outcome	Effect of variable	Analysis type	Study	n	Birth year of cohort	Reference
Autoimmune, systemic lupus erythematosus	Earlier menopause → increased risk systemic lupus erythematosus	Trend of increased risk of systemic lupus erythematosus with younger age at menopause, p<0.05	Cox PH model adjusted for age, smoking, birthweight, body size as child.	Nurses' Health Study, USA; Nurses' Health Study II, USA	238,308	1921-1946 (aged 30-55 years in 1976); 1947-1964 (aged 25-42 years in 1989)	Costenbader, Feskanich et al. Reproductive and menopausal factors and risk of systemic lupus erythematosus in women. *Arthritis Rheum*. **56**, 1251-1262.(2007)
Bone, bone mineral density	Earlier menopause → decreased bone density	Increase in BMD with increasing ANM. Wrist (beta=0.002, p<0.01), radius (beta=0.003, p<0.001), spine (beta=0.005, p<0.001), hip (beta=0.004, p<0.05).	Multiple regression of age at natural menopause. Adjusted for age, obesity, oestrogen use, oral contraceptive use, number pregnancies, smoking, thiazide use, type pf menopause.	California, USA	742	1899-1931 (aged 60-89 in 1988-1991)	Kritz-Silverstein and Barrett-Connor. Early menopause, number of reproductive years, and bone mineral density in postmenopausal women. *Am J Public Health*. **83**, 983-988.(1993)
Bone, fractures	Early menopause - > increased bone fractures	EM associated with fractures during lifetime OR=1.4 (95% CI 1.1–1.7), after menopause OR=1.4 (95% CI 1.0–1.8) and after age 50 years OR=2.1 (95% CI 1.6–2.7). Overall risk of fractures OR=1.5 (CI 1.2–1.8)	EM<45 years. Odds ratio adjusted for age, BMI, bone mineral density, weight, smoking habits, use of hormones.	Population based, The Netherlands	4,725	1912-1944 (aged 50-80 years in 1992-1994)	van Der Voort, van Der Weijer et al. Early menopause: increased fracture risk at older age. *Osteoporos Int*. **14**, 525-530.(2003)
Bone, fractures	Early menopause → increased fractures	Menopausal at baseline, menopause<47 years fracture risk =1.76 (95% CI 1.15-2.70), p<0.01 (univariate); borderline in multivariate RR=1.49 (95% CI 0.97-2.29) p=0.07.	EM before 47 years; Cox PH univariate and adjusted for body weight, BMI, forearm BMD, strength index, menopause status, physical activity.	Prospective population-based observational study, Malmo, Sweden; followed 1977-2011	390	1929 (white north Europeans aged 48 in 1977)	Svejme, Ahlborg et al. Low BMD is an independent predictor of fracture and early menopause of mortality in post-menopausal women--a 34-year prospective study. *Maturitas*. **74**, 341-345.(2013)
Bone, fractures	Early menopause → increased fragility fracture	Menopause <47 years, fragility fracture RR=1.68 (95% CI 1.05–2.57)	EM before 47 years, risk at age 77; Cox PH univariate and multivariate (adjusted for body weight, BMI, forearm BMD, strength index, menopause status, physical activity).	Prospective population-based observational study, Malmo, Sweden; followed 1977-2011	390	1929 (white north Europeans aged 48 in 1977)	Svejme, Ahlborg et al. Early menopause and risk of osteoporosis, fracture and mortality: a 34-year prospective observational study in 390 women. *Bjog*. **119**, 810-816.(2012)
Bone, fractures	Early menopause → increased risk of hip fracture	Menopause <45 years vs 50+ years, RR=1.22 (95% CI 1.05–1.40)	Cox PH adjusted for age, region, SES, BMI, cigarette smoking, alcohol consumption, physical activity, oral contraceptive use, parity, and medical history.	Million Women Study, UK	419,723	Recruited 1999-2004	Banks, Reeves et al. Hip fracture incidence in relation to age, menopausal status, and age at menopause: prospective analysis. *PLoS Med*. **6**, e1000181.(2009)

Outcome	Effect on outcome	Effect of variable	Analysis type	Study	n	Birth year of cohort	Reference
Bone, osteoporosis	Earlier menopause → increased odds osteoporosis	Increasing age at menopause associated with decreased odds osteoporosis, OR=0.97 (95% CI 0.94-0.997) p=0.029	Multinomial logistic regression including menopausal age, duration of menopause, parity, smoking, glucose, total cholesterol, triglycerides, LDL-c, VLDL-c, HDL-c, presence of systemic hypertension and diabetes mellitus	Study at Ankara Etlik Maternity and Women's Health Teaching Hospital, Turkey	2,769	Aged 40-63 years	Demir, Haberal et al. Identification of the risk factors for osteoporosis among postmenopausal women. *Maturitas*. **60**, 253-256.(2008)
Bone, osteoporosis	Early menopause → increased osteoporosis	For menopause <47 years, osteoporosis RR=1.83 (95% CI 1.22–2.74)	EM before 47 years, risk at age 77; Cox PH univariate and multivariate (adjusted for body weight, BMI, forearm BMD, strength index, menopause status, physical activity).	Prospective population-based observational study, Malmo, Sweden; followed 1977-2011	390	1929 (white north Europeans aged 48 in 1977)	Svejme, Ahlborg et al. Early menopause and risk of osteoporosis, fracture and mortality: a 34-year prospective observational study in 390 women. *Bjog*. **119**, 810-816.(2012)
Cancer, bladder	Earlier menopause → increased risk of bladder cancer	Compared with menopause at age 48, menopause 43-47 years HR=1.32 (95% CI 0.90-1.94), <42 years HR=1.60 (95% CI 1.06–2.39). p-trend=0.02. Similar for natural and surgical menopause.	Cox PH adjusted for age and smoking.	Iowa Women's Health Study, USA	37,459	1917-1931 (aged 55-69 in 1986)	Prizment, Anderson et al. Reproductive risk factors for incident bladder cancer: Iowa Women's Health Study. *Int J Cancer*. **120**, 1093-1098.(2007)
Cancer, bladder	Early menopause → increased risk of bladder cancer	Menopause ≤45 years vs 50+ years, incidence of bladder cancer IRR=1.63 (95% CI 1.20-2.223), modified by smoking (p for interaction = 0.01)	Cox PH adjusted for BMI, smoking. Surgical and natural menopause included.	Nurses' Health Study, USA	84,330	1921-1946 (aged 30-55 years in 1976)	McGrath, Michaud et al. Hormonal and reproductive factors and the risk of bladder cancer in women. *Am J Epidemiol*. **163**, 236-244.(2006)
Cancer, breast	Later menopause - > increased risk ductal breast cancer	Per year increase in menopause age, for ductal tumours HR=1.01 (95% CI 1.00-1.01), for hormone receptor positive ductal tumours HR=1.01 (95% CI 1.00-1.01).	Cox PH adjusted for age, age at menarche, parity, age at first birth, postmenopausal hormone use, type of menopause, BMI, BMI at age 18, alcohol, family history of breast cancer, history benign breast disease	Nurses' Health Study, USA	92,468	1921-1946 (aged 30-55 years in 1976)	Kotsopoulos, Chen et al. Risk factors for ductal and lobular breast cancer: results from the nurses' health study. *Breast Cancer Res*. **12**, R106.(2010)
Cancer, breast	Later menopause - > increased risk lobular breast cancer	Per year increase in menopause age, for lobular tumours HR=1.01 (95% CI 1.00-1.02), for hormone receptor positive ductal tumours HR=1.02 (95% CI 1.00-1.03).	Cox PH adjusted for age, age at menarche, parity, age at first birth, postmenopausal hormone use, type of menopause, BMI, BMI at age 18, alcohol, family history of breast cancer, history benign breast disease	Nurses' Health Study, USA	92,468	1921-1946 (aged 30-55 years in 1976)	Kotsopoulos, Chen et al. Risk factors for ductal and lobular breast cancer: results from the nurses' health study. *Breast Cancer Res*. **12**, R106.(2010)

Outcome	Effect on outcome	Effect of variable	Analysis type	Study	n	Birth year of cohort	Reference
Cancer, breast	Later menopause → increased breast cancer risk	Breast cancer risk per yr older age at menopause, OR=1.029 (95% CI 1.025–1.032; p<0.0001). For premenopausal women of 45-54 years vs postmenopausal, breast cancer RR=1.43 (95% 1.33-1.52, p<0.001) . Stronger for lobular than ductal tumours (p<0.006). Associations attenuated by increasing adiposity. Stronger for oestrogen receptor positive than negative (p<0.01)	Conditional logistic regression, adjusted for stratified by study, by age at diagnosis, year of birth, parity, age at first birth, smoking, alcohol consumption, height, and current BMI	117 studies	118,964 cases, 306,091 controls		Menarche, menopause, and breast cancer risk: individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. *Lancet Oncol*. **13**, 1141-1151.(2012)
Cancer, breast	Later menopause → increased breast cancer risk	Compared with natural menopause at 45-54 years, menopause at 55+ years RR=1.48, menopause <45 years RR=0.73; risk greatest after age 70 years.	Compared observed cancer cases from cancer registry to expected from national sample using Mantel Haenszel.	Connecticut Cancer Registry, USA; National Health Examination Survey of 1960-62, USA	3887 cases, 3581 in national sample.	Aged under 80 years in 1972	Trichopoulos, MacMahon et al. Menopause and breast cancer risk. *J Natl Cancer Inst*. **48**, 605-613.(1972)
Cancer, breast	Later menopause → increased risk of ductal carcinoma in situ	Increasing age at menopause associated with increased risk of ductal carcinoma in situ, for menopause at 55+ vs 45-54 years, HR=1.39 (95% CI 1.08–1.79)	Cox PH adjusted for age, age at first live birth, parity, use of hormone therapy, breast biopsy, breast cancer in 1st degree relative, mammogram in past 2 years, education	Women's Health Initiative, USA	64,060	1914-1948 (aged 50-79 years 1993-1998)	Kabat, Kim et al. Reproductive and menstrual factors and risk of ductal carcinoma in situ of the breast in a cohort of postmenopausal women. *Cancer Causes Control*. **22**, 1415-1424.(2011)
Cancer, breast	POI → reduced risk of breast cancer	For breast cancer HR=0.59 (95% CI 0.38-0.91). Similar when HRT users excluded. Note: 73% of POF cases were natural.	Logistic regression adjusted for age, income, occupation, BMI, waist hip ratio, smoking, nulliparity, age at menarche, type of menopause and HRT use.	Shanghai Women's Health Study, China	1003 cases POI	1926-1960 (40-70 years 1996-2000)	Wu, Cai et al. Impact of premature ovarian failure on mortality and morbidity among Chinese women. *PLoS One*. **9**, e89597.(2014)
Cancer, colorectal	Later menopause → increased colorectal cancer	Risk of colorectal cancer increased with older age at menopause: menopause <40 (ref), 40-44yrs HR=1.20 (95% CI 1.01-1.42), 45-49 years HR=1.15 (95% CI 0.98-1.35), 50-54 years HR=1.13 (95% CI 0.97-1.31), 55+ years HR=1.50 (95% CI 1.23-1.83). P-trend=0.008.	Cox PH adjusted for age, BMI, education level, alcohol consumption, family history of colorectal cancer, race, smoking history, diabetes, physical activity level, and use of hormone therapy.	National Institutes of Health–American Association of Retired Persons Diet and Health study, USA	214,162 postmenopausal women	1924-1946 (aged 50-71 in 1995-1996)	Zervoudakis, Strickler et al. Reproductive history and risk of colorectal cancer in postmenopausal women. *J Natl Cancer Inst*. **103**, 826-834.(2011)

Outcome	Effect on outcome	Effect of variable	Analysis type	Study	n	Birth year of cohort	Reference
Cancer, endometrial	Later menopause → increased risk of endometrial cancer	Compared with menopause <=50 years, 51-52 years HR=1.32 (95% CI 1.04–1.68), 53-55 years HR=1.49 (95% CI 1.18–1.89), >55 years HR=2.20 (95% CI 1.61–3.01). P<0.0001 for trend.	Menopause <=50, 51-52, 53-55, >55 years. Cox PH adjusted for age, centre, BMI, physical activity, alcohol consumption, diabetes, smoking status, education level.	European Prospective Investigation into Cancer and Nutrition (EPIC).	1017 cases of endometrial cancer, 301601 controls.	1922-1965 (aged 35-70, 1992-2000)	Dossus, Allen et al. Reproductive risk factors and endometrial cancer: the European Prospective Investigation into Cancer and Nutrition. *Int J Cancer*. **127**, 442-451.(2010)
Cancer, endometrial	Later menopause → increased risk of endometrial cancer	Increasing menopause age increased risk of endometrial cancer (P-trend = 0.0003).	Cox PH adjusted for age, parity, age at first birth, age at last birth, oral contraceptive duration, postmenopausal hormone use, type of menopause, BMI, smoking, diabetes, family history of endometrial cancer, age at menarche.	Nurses' Health Study, USA	95,638	1921-1946 (aged 30-55 years in 1976)	Karageorgi, Hankinson et al. Reproductive factors and postmenopausal hormone use in relation to endometrial cancer risk in the Nurses' Health Study cohort 1976-2004. *Int J Cancer*. **126**, 208-216.(2010)
Cancer, lung cancer	Earlier menopause → increased risk lung cancer	Menopause at 51+ years vs <46 years, risk of lung cancer in non-smokers HR=0.63 (95% CI 0.40-1.00)	Cox PH in non-smokers adjusted for birth cohort, passive smoking	Shanghai Women's Health Study, China	71,314	1926-1960 (40-70 years 1996-2000)	Weiss, Lacey et al. Menstrual and reproductive factors in association with lung cancer in female lifetime nonsmokers. *Am J Epidemiol*. **168**, 1319-1325.(2008)
Cancer, lung cancer	Early menopause → increased lung cancer risk	Menopause <44 years vs 48-49 years, lung cancer HR=1.39 (95%CI 1.14-1.70). Decreasing risk of lung cancer with increasing menopause age P=0.0004 for trend. Trend not sign. In never and former smoker, p=0.001 for current smokers. In smokers, menopause <44 years vs 48-49 years, lung cancer HR=1.79 (95%CI 1.32-2.34).	Cox PH adjusted for age at menarche, parity, type of menopause, PMH use, OCP use, smoking status, age at start smoking, cigarettes per day, time since quitting, fruit/vegetable intake, BMI, environmental smoking exposure	Nurses' Health Study, USA	107,171 postmenopausal women	1921-1946 (aged 30-55 years in 1976)	Baik, Strauss et al. Reproductive factors, hormone use, and risk for lung cancer in postmenopausal women, the Nurses' Health Study. *Cancer Epidemiol Biomarkers Prev*. **19**, 2525-2533.(2010)
Cancer, ovarian	Later menopause → increased risk of ovarian cancer	Age at natural menopause 50+ years vs <45 years, RR=2.6 (95% CI 1.1-6.1), chi-squared test for trend p<0.01	Relative risk adjusted for age, social class, gravidity, contraceptive use, hysterectomy	Hospital based London and Oxford, UK	235 cases, 451 controls	Under 65 in 1978-1983	Booth, Beral et al. Risk factors for ovarian cancer: a case-control study. *Br J Cancer*. **60**, 592-598.(1989)
Cancer, pancreatic	Early menopause → increased risk pancreatic cancer	Compared with menopause <45 years, menopause 45-49 years HR=0.61 (95% CI 0.40-0.94), 50-54 years HR=0.75 (95% CI 0.51-1.09), 55+ years HR=0.35 (95% CI 0.18-0.68) (P trend=0.005). Similar for natural and surgical menopause.	Cox PH adjusted for age, smoking, diabetes, multivitamin use, HRT use	Iowa Women's Health Study, USA	37,459	1917-1931 (aged 55-69 in 1986)	Prizment, Anderson et al. Pancreatic cancer incidence in relation to female reproductive factors: Iowa Women's Health Study. *Jop*. **8**, 16-27.(2007)

Outcome	Effect on outcome	Effect of variable	Analysis type	Study	n	Birth year of cohort	Reference
Cancer, upper GI tract	Earlier menopause → increased risk of squamous cell carcinoma of the upper GI tract	Older age at menopause associated inversely with squamous cell carcinoma (p-trend across categories=0.013) .	Cox PH adjusted for education, alcohol, BMI, smoking, physical activity, age, fruit intake, vegetable intake, total energy intake.	National Institutes of Health–American Association of Retired Persons Diet and Health study, USA	125,887	1924-1946 (aged 50-71 in 1995-1996)	Freedman, Lacey et al. The association of menstrual and reproductive factors with upper gastrointestinal tract cancers in the NIH-AARP cohort. *Cancer*. **116**, 1572-1581.(2010)
Cognitive function	Early menopause → increased odds of cognitive impairment	Did not distinguish between natural and surgical menopause. Compared with menopause at 50-54 years, cognitive impairment associated with menopause <40 years, HR=1.64 (95% CI 1.03-2.61); menopause 40-44 years HR=1.39 (95% CI 1.08-1.78). In co-twin analysis, menopause <44 years HR=2.64 (95% CI 1.32-5.30).	Case-control and matched co-twin control, logistic regression, adjusted for age and education.	HARMONY study, Sweden	1451 twin pairs and 3702 singletons	Aged 65-84 years in 1998 onwards.	Rasgon, Magnusson et al. Endogenous and exogenous hormone exposure and risk of cognitive impairment in Swedish twins: a preliminary study. *Psychoneuroendocrinology*. **30**, 558-567.(2005)
Cognitive function	POI → long-term reduced cognitive function	POI associated with reduced verbal fluency HR=2.24 (95% CI 1.44–3.48) p=0.0004; reduced visual memory HR=1.77 (95% CI 1.16–2.72) p=0.009; decline in psychomotor speed over 7 yr period, HR=1.36 (95% CI 1.09–1.71) p=0.01.	Cox PH adjusted for recruitment centre, age, education level, physical limitations, chronic illness, depression, use of HT at the menopause and current HT use.	French Three-City Study.	4,868	Aged 65+ years in 1999-2001	Ryan, Scali et al. Impact of a premature menopause on cognitive function in later life. *Bjog*. **121**, 1729-1739.(2014)
CVD	Being menopausal → increased CVD	Menopause status associated with premature CVD, OR=2.82 (95% CI 1.91–4.19)	Multivariate logistic regression including covariates for diabetes, hypercholesterolaemia, smoking (current and former), hypertension, low leisure-time physical activity, parental CVD, WHR, HDL-cholesterol level, triglycerides, biomarkers, menopause status, early/late post menopause.	PRECADIW study, Poland	323 cases, 347 controls	1950- (aged	Lubiszewska, Kruk et al. The impact of early menopause on risk of coronary artery disease (PREmature Coronary Artery Disease In Women--PRECADIW case-control study). *Eur J Prev Cardiol*. **19**, 95-101.(2012)
CVD	Earlier menopause → increased risk of CVD in women with RA	Natural or artificial menopause <45 years in women with RA, HR=1.56 (95% CI 1.08-2.26)	Cox PH	Population based cohort of women with RA	600	1910-1962; Data collected 1955-2007, aged 45+ at diagnosis	Pfeifer, Crowson et al. The influence of early menopause on cardiovascular risk in women with rheumatoid arthritis. *J Rheumatol*. **41**, 1270-1275.(2014)

Outcome	Effect on outcome	Effect of variable	Analysis type	Study	n	Birth year of cohort	Reference
CVD, atherosclerosis	Earlier menopause → increased atherosclerosis	Compared with menopause at 35-48 years, 49-51 years HR=0.84 (95% CI 0.69-1.03), 52-53 years HR=0.67 (95% Ci 0.53-0.86), 54-60 years HR=0.73 (95% CI 0.56-0.96). P-trend=0.001. Excluding surgical menopause did not change results.	ANCOVA, menopause age grouped as 35-48 years, 49-51 years, 52-53 years, 54-60 years. Adjusted for use of oestrogen, smoking before menopause, BMI, and length of education	Trømso health survey, Norway	2,588	1920-1940 (aged 55-74 years in 1994/5)	Joakimsen, Bonaa et al. Population-based study of age at menopause and ultrasound assessed carotid atherosclerosis: The Tromso Study. *J Clin Epidemiol*. **53**, 525-530.(2000)
CVD, cholesterol levels	Early menopause → increased odds hypercholesterolaemia	EM (<45 years) associated with increased odds of hypercholesterolaemia (OR=2.72 95% CI 1.93-3.82)	Multiple logistic regression adjusting for age, BMI, HRT, smoking, alcohol drinking, physical activity.	Japan Nurses' Health Study	22,426	1942-1961 (aged 40-59 years in 2001)	Lee, Hayashi et al. Independent association between age at natural menopause and hypercholesterolemia, hypertension, and diabetes mellitus: Japan nurses' health study. *J Atheroscler Thromb*. **20**, 161-169.(2013)
CVD, coronary heart disease	Early menopause → increased coronary heart disease	Adjusted model for CHD, HR=2.08 (95% CI 1.17-3.70)	EM natural or surgical before 46 years, Cox PH adjusted for age, race/ethnicity, study site, CVD risk factors.	Multi-Ethnic Study of Atherosclerosis, USA	2509 (987 White, 331 Chinese, 641 Black, 550 Hispanic)	1916-1957 (45 to 84 years in 2000–2002)	Wellons, Ouyang et al. Early menopause predicts future coronary heart disease and stroke: the Multi-Ethnic Study of Atherosclerosis. *Menopause*. **19**, 1081-1087.(2012)
CVD, coronary heart disease	Early menopause → increased ischaemic heart disease	Risk of ischaemic heart disease for menopause <40 years, HR=2.2 (95% CI 1.0–4.9)	Multivariate Cox PH models were fitted with smoking, hypertension, self rated health, body-mass index, angina, diabetes and use of HT.	Survey of Danish Nurse Association	10,533	Over 44 years in 1993	Lokkegaard, Jovanovic et al. The association between early menopause and risk of ischaemic heart disease: influence of Hormone Therapy. *Maturitas*. **53**, 226-233.(2006)
CVD, coronary heart disease	Early menopause → increased risk of coronary heart disease	Overall, per 1 yr decrease in ANM, risk of coronary heart disease RR=1.03 (95% CI 1.01-1.05); in smokers RR=1.04 (95% CI 1.01-1.07); not significant in never smokers.	Logistic regression adjusted for age, age at menarche, parity, smoking status, parental history of premature myocardial infarction, history of diabetes, history of hypertension, history of hypercholesterolaemia.	Nurses' Health Study, USA	35,616	1921-1946 (aged 30-55 years in 1976)	Hu, Grodstein et al. Age at natural menopause and risk of cardiovascular disease. *Arch Intern Med*. **159**, 1061-1066.(1999)
CVD, coronary heart disease	Early menopause → possible increased coronary heart disease	Women aged 40-64 years, menopause <49 years associated with increased coronary heart disease compared with menopause 49+ years, HR=1.85 (95% CI 0.92-3.73, p = 0.08)	Cox PH adjusted for smoking status, alcohol intake, marital status, type of menopause, education, hypertension, diabetes. Stratified by age at baseline.	Japan Collaborative Cohort (JACC) Study	37,965	1909-1950 (aged 40-79 years in 1988-1990)	Cui, Iso et al. Relationships of age at menarche and menopause, and reproductive year with mortality from cardiovascular disease in Japanese postmenopausal women: the JACC study. *J Epidemiol*. **16**, 177-184.(2006)

Outcome	Effect on outcome	Effect of variable	Analysis type	Study	n	Birth year of cohort	Reference
CVD, deep vein thrombosis	Early and late menopause → increased risk of DVT	Increased DVT risk for menopause <40 years (HR=1.8, 95%CI 1.2–2.8) and >55 years (HR=1.6, 95%CI 1.0–2.7)	Cox PH adjusted for treatment, age, BMI, race, history of events, smoking, MET-score, hormone use.	Women's Health Initiative Hormone Therapy Trials, USA	294	1924-1948 (aged 50-79 in 1993-1998)	Canonico, Plu-Bureau et al. Age at menopause, reproductive history, and venous thromboembolism risk among postmenopausal women: the Women's Health Initiative Hormone Therapy clinical trials. *Menopause*. **21**, 214-220.(2014)
CVD, heart failure	Early menopause → increased heart failure	EM increased risk of incident heart failure HR=1.66 (95% CI 1.01-2.73), per year increase in age at menopause decreased risk of incident heart failure by HR=0.96 (95% CI 0.94-0.99).	Aged 45-85 years without CVD, early menopause aged<45 years, multivariate Cox PH adjusted for age, ethnicity, centre, reproductive characteristics, educational status, smoking, hypertension, diabetes, kidney function, BMI, LVH, insulin resistance, inflammation and interim MI.	Multi-Ethnic Study of Atherosclerosis, USA	2,947	1916-1957 (45 to 84 years in 2000–2002)	Ebong, Watson et al. Age at menopause and incident heart failure: the Multi-Ethnic Study of Atherosclerosis. *Menopause*. **21**, 585-591.(2014)
CVD, hypertension	POI → reduced risk of hypertension	Note: 73% of POF cases were natural. For hypertension HR=0.84 (95% CI 0.73-0.97). Similar when HRT users excluded.	Logistic regression adjusted for age, income, occupation, BMI, waist hip ratio, smoking, nulliparity, age at menarche, type of menopause and HRT use.	Shanghai Women's Health Study, China	1003 cases POI	1926-1960 (40-70 years 1996-2000)	Wu, Cai et al. Impact of premature ovarian failure on mortality and morbidity among Chinese women. *PLoS One*. **9**, e89597.(2014)
CVD, NT-proBNP levels	Early menopause → increased N-terminal pro brain natriuretic peptide (NT-proBNP) levels (marker for CVD and heart failure)	EM 10.7% increase in NT-proBNP levels, each 1-year increase in menopause age associated with 0.7% decrease in NT-proBNP levels	Aged 45-85 years without CVD, early menopause aged<45 years, multivariate linear regression	Multi-Ethnic Study of Atherosclerosis, USA	2,275	1916-1957 (45 to 84 years in 2000–2002)	Ebong, Watson et al. Association of menopause age and N-terminal pro brain natriuretic peptide: the Multi-Ethnic Study of Atherosclerosis. *Menopause*. **22**, 527-533.(2015)
CVD, stroke	Early menopause - > increased stroke	Adjusted model for stroke, HR=2.19 (95% CI 1.11-4.32)	EM natural or surgical before 46 years, Cox PH adjusted for age, race/ethnicity, study site, CVD risk factors.	Multi-Ethnic Study of Atherosclerosis, USA	2509 (987 White, 331 Chinese, 641 Black, 550 Hispanic)	1916-1957 (45 to 84 years in 2000–2002)	Wellons, Ouyang et al. Early menopause predicts future coronary heart disease and stroke: the Multi-Ethnic Study of Atherosclerosis. *Menopause*. **19**, 1081-1087.(2012)
CVD, stroke	POI → increased risk of cerebral infarction	Risk of cerebral infarction, menopause <40 years vs 50-54 years, HR=2.57 (95% CI 1.20-5.49).	Cox PH adjusted for age, systolic blood pressure, total cholesterol, body mass index, smoking habits, and alcohol drinking habits.	Jichi Medical School Cohort Study, Japan	4,790	1903-1959 (aged 36-89 years in 1992-1995)	Baba, Ishikawa et al. Premature menopause is associated with increased risk of cerebral infarction in Japanese women. *Menopause*. **17**, 506-510.(2010)

Outcome	Effect on outcome	Effect of variable	Analysis type	Study	n	Birth year of cohort	Reference
CVD, venous thromboembolism	Earlier menopause → increased risk VTE	Age at menopause 55+ vs <45 years associated with reduced risk of VTE, HR=0.74 (95% CI 0.59-0.93)	Cox PH adjusted for age, BMI, education, physical activity.	Iowa Women's Health Study, USA	41,836	1917-1931 (aged 55-69 in 1986)	Lutsey, Virnig et al. Correlates and consequences of venous thromboembolism: The Iowa Women's Health Study. *Am J Public Health*. **100**, 1506-1513.(2010)
CVD, venous thromboembolism	Early and late menopause → increased risk of VTE	Increased VTE risk for menopause <40 years (HR=1.8, 95%CI 1.2–2.7) and >55 years (HR=1.5, 95%CI 1.0–2.4)	Cox PH adjusted for treatment, age, BMI, race, history of events, smoking, MET-score, hormone use.	Women's Health Initiative Hormone Therapy Trials, USA	294	1924-1948 (aged 50-79 in 1993-1998)	Canonico, Plu-Bureau et al. Age at menopause, reproductive history, and venous thromboembolism risk among postmenopausal women: the Women's Health Initiative Hormone Therapy clinical trials. *Menopause*. **21**, 214-220.(2014)
Depression	Early menopause → increased risk of depression	Menopause at 52+ years vs <46 years, reduced risk of depression (OR=0.35, 95%CI 0.22–0.55)	Multi-variate logistic regression adjusted for menarche age, menopause age, menopause status, type of menopause, first pregnancy age, pregnancy number, first birth age, last birth age, breast feeding duration, OC usage, duration OC, HRT use	Korean National Health and Nutrition Examination Survey (KNHANES) V	4,869	>19 years in 2010-2012	Jung, Shin et al. Hormone-related factors and post-menopausal onset depression: results from KNHANES (2010-2012). *J Affect Disord*. **175**, 176-183.(2015)
Depression	POI → higher prevalence of depression in lifetime	Lifetime history of depression higher in women with POI (p<0.001).	Compared prevalence of depression with published prevalences from large community-based studies, primary care and gynaecology clinic-based studies.		174		Schmidt, Luff et al. Depression in women with spontaneous 46, XX primary ovarian insufficiency. *J Clin Endocrinol Metab*. **96**, E278-287.(2011)
Diabetes, dysglycaemia	Post-menopausal → higher odds dysglycaemia	In women aged <50yrs who were post-menopausal, for dysglycaemia OR=1.50 (95% CI 1.18-1.91).	Logistic regression adjusted for age.	Toranomon Hospital Health Management Centre Study, Japan	6,308 premenopausal and 4,570 postmenopausal	Data collected 1997-2007	Heianza, Arase et al. Effect of postmenopausal status and age at menopause on type 2 diabetes and prediabetes in Japanese individuals: Toranomon Hospital Health Management Center Study 17 (TOPICS 17). *Diabetes Care*. **36**, 4007-4014.(2013)
Diabetes, pre-diabetes	Post-menopausal → higher odds pre-diabetes	For natural menopause, age-adjusted odds pre-diabetes OR=1.33 (95% CI 1.2-1.48).	Logistic regression adjusted for age.	Toranomon Hospital Health Management Centre Study, Japan	6,308 premenopausal and 4,570 postmenopausal	Data collected 1997-2007	Heianza, Arase et al. Effect of postmenopausal status and age at menopause on type 2 diabetes and prediabetes in Japanese individuals: Toranomon Hospital Health Management Center Study 17 (TOPICS 17). *Diabetes Care*. **36**, 4007-4014.(2013)

Outcome	Effect on outcome	Effect of variable	Analysis type	Study	n	Birth year of cohort	Reference
Diabetes, type II	Earlier menopause → increased risk of Type II diabetes	Menopause under 40 years compared with 45-49 years, risk of type 2 diabetes, HR=1.32 (95% CI 1.04–1.69)	Cox PH adjusted for age, BMI, smoking status, alcohol consumption, education level, physical activity, number of full-term pregnancies, oral contraceptive and HRT use.	InterAct, Europe	3691 cases, 4408 subcohort members	Data collected 1991-2007, mean age at entry 59.2 years.	Brand, van der Schouw et al. Age at menopause, reproductive life span, and type 2 diabetes risk: results from the EPIC-InterAct study. *Diabetes Care*. **36**, 1012-1019.(2013)
Diabetes, type II	Post-menopausal → higher odds diabetes	For natural menopause, age-adjusted odds diabetes OR=1.4 (95% CI 1.03-1.89).	Logistic regression adjusted for age.	Toranomon Hospital Health Management Centre Study, Japan	6,308 premenopausal and 4,570 postmenopausal	Data collected 1997-2007	Heianza, Arase et al. Effect of postmenopausal status and age at menopause on type 2 diabetes and prediabetes in Japanese individuals: Toranomon Hospital Health Management Center Study 17 (TOPICS 17). *Diabetes Care*. **36**, 4007-4014.(2013)
Glaucoma	Earlier menopause → increased risk of glaucoma	In postmenopausal women aged 65+ years, menopause at >54 years vs 50-54 years HR=0.53 (95% CI 0.32-0.89)	Multivariable Cox PH	Nurses' Health Study, USA	66,417	1921-1946 (aged 30-55 years in 1976)	Pasquale, Rosner et al. Attributes of female reproductive aging and their relation to primary open-angle glaucoma: a prospective study. *J Glaucoma*. **16**, 598-605.(2007)
Gout	Earlier menopause → increased risk of gout	Menopause <45 years vs 50-54 years associated with increased gout, RR=1.62 (95% CI 1.12-2.33)	Cox PH adjusted for age, smoking, BMI, use of diuretics, hypertension, alcohol, meat intake, seafood intake, dairy intake, coffee intake, fructose intake vitamin C intake, total energy intake.	Nurses' Health Study, USA	92,535	1921-1946 (aged 30-55 years in 1976)	Hak, Curhan et al. Menopause, postmenopausal hormone use and risk of incident gout. *Ann Rheum Dis*. **69**, 1305-1309.(2010)
Low back pain	Early menopause → increased odds of low back pain	Decreasing age at menopause associated with more low back pain, p=0.005. For women with menopause <30 years, OR=3.2 (95% CI 1.8-5.4)	Multivariate logistic regression including age, education, number of pregnancies, physical activity, BMI, work.	National Health and Nutrition Examination survey II, USA	5,325	Aged 25+ years in 1976-1980	Adera, Deyo et al. Premature menopause and low back pain. A population-based study. *Ann Epidemiol*. **4**, 416-422.(1994)
Mortality, all causes	Early menopause → increased all cause mortality	Menopause age in categories of 35–40 years, 41–44 years, 45–48 years, 49–51 years (reference), 52–55 years, and 56–60 years. P=<0.001 for linear trend in total mortality, decreasing with menopause age.	Cox PH adjusted for diabetes, hypertension, parity, age at first birth, and physical activity in leisure.	Adventist Health Study, California, USA	5,279	pre-1951 (25 and older in 1976 at recruitment)	Jacobsen, Knutsen et al. Age at natural menopause and total mortality and mortality from ischemic heart disease: the Adventist Health Study. *J Clin Epidemiol*. **52**, 303-307.(1999)
Mortality, all causes	Early menopause → increased all cause mortality	Menopause <40 years, HR=1.34 (95% CI 0.96-1.84) vs menopause at 50-54 years; P-trend=0.04.	Cox PH adjusted for marital status, education, age at menarche, diet, BMI, smoking, alcohol, physical activity, parity, age at first birth, lactation, oral contraceptive use, unilateral oophorectomy.	Black Women Heath Study, USA	11,212	1926-1974 (aged 21-69 in 1995)	Li, Rosenberg et al. Age at natural menopause in relation to all-cause and cause-specific mortality in a follow-up study of US black women. *Maturitas*. **75**, 246-252.(2013)

Outcome	Effect on outcome	Effect of variable	Analysis type	Study	n	Birth year of cohort	Reference
Mortality, all causes	Early menopause → increased all cause mortality	All cause mortality higher in menopause 40-44 years vs 50-54 years (RR=1.04 (95% CI 1.00-1.08))	20 years follow up. Excluded women ever taking HRT or ever smoking. Cox PH adjusted for age, race, marital status, BMI, age at menarche, parity, educational level, alcohol comsumption, oral contraceptive use, exercise.	Cancer Prevention Study II	68,154	Aged 30+ years in 1982.	Mondul, Rodriguez et al. Age at natural menopause and cause-specific mortality. *Am J Epidemiol*. **162**, 1089-1097.(2005)
Mortality, all causes	Early menopause → increased all cause mortality	Per year delayed menopause, for mortality from all causes HR=0.92 (95% CI 0.88-0.96)	Cox PH adjusted for age, type of menopause, parity, age at first birth, BMI, smoking, hypertensions, diabetes, previous CVD	Diagnostisch Onderzoek Mammacarcinoom, Utrecht, The Netherlands	12,134	Aged 48-68 in 1974-1977	Ossewaarde, Bots et al. Age at menopause, cause-specific mortality and total life expectancy. *Epidemiology*. **16**, 556-562.(2005)
Mortality, all causes	Early menopause → increased all cause mortality	1.6% reduced mortality per 3 years increased age at menopause (95% CI 0.6-2.7%). Stronger in women who attained age of <70 years vs 80+ years.	Cox PH adjusted for birth cohort, county, occupational group. Analyses in three predetermined age groups: <70 years, 70–79 years, and 80 years or above.	Norway	19,731	1886-1926	Jacobsen, Heuch et al. Age at natural menopause and all-cause mortality: a 37-year follow-up of 19,731 Norwegian women. *Am J Epidemiol*. **157**, 923-929.(2003)
Mortality, all causes	Early menopause → increased all cause mortality	Menopausal at baseline mortality risk =1.72 (95% CI 1.16-2.54), p=0.01 (univariate), 1.62 (95% CI 1.09-2.39) p=0.02 in multivariate.	EM before 47 years; Cox PH univariate and multivariate (adjusted for body weight, BMI, forearm BMD, strength index, menopause status, physical activity).	Prospective population-based observational study, Malmo, Sweden; followed 1977-2011	390	1929 (white north Europeans aged 48 in 1977)	Svejme, Ahlborg et al. Low BMD is an independent predictor of fracture and early menopause of mortality in post-menopausal women--a 34-year prospective study. *Maturitas*. **74**, 341-345.(2013)
Mortality, all causes	Early menopause → increased all cause mortality	Menopause <47 years, mortality RR=1.59 (95% CI 1.04–2.36)	EM before 47 years, risk at age 77; Cox PH univariate and multivariate (adjusted for body weight, BMI, forearm BMD, strength index, menopause status, physical activity).	Prospective population-based observational study, Malmo, Sweden; followed 1977-2011	390	1929 (white north Europeans aged 48 in 1977)	Svejme, Ahlborg et al. Early menopause and risk of osteoporosis, fracture and mortality: a 34-year prospective observational study in 390 women. *Bjog*. **119**, 810-816.(2012)

Outcome	Effect on outcome	Effect of variable	Analysis type	Study	n	Birth year of cohort	Reference
Mortality, all causes	Early menopause → increased all cause mortality	HR decreased from HR=1.16 (95% CI 1.04-1.29) to HR=0.99(95% CI 0.88-1.11) from youngest to oldest menopause age, p-trend=0.02	Quintiles of age at menopause, tested for p-trend. Cox PH (outcome death) adjusted for age, education, income, occupation, marital status, BMI, waist hip ratio, smoking, alcohol, number live births, age at menarche.	Shanghai Women's Health Study, China	31,995	1926-1960 (40-70 years 1996-2000)	Wu, Cai et al. Age at menarche and natural menopause and number of reproductive years in association with mortality: results from a median follow-up of 11.2 years among 31,955 naturally menopausal Chinese women. *PLoS One*. **9**, e103673.(2014)
Mortality, all causes	POI → increased mortality from all causes	Note: 73% of POF cases were natural. For mortality all causes, HR=1.28(95% CI 1.07-1.53). Same when HRT users excluded.	Logistic regression adjusted for age, income, occupation, BMI, waist hip ratio, smoking, nulliparity, age at menarche, type of menopause and HRT use.	Shanghai Women's Health Study, China	1003 cases POI	1926-1960 (40-70 years 1996-2000)	Wu, Cai et al. Impact of premature ovarian failure on mortality and morbidity among Chinese women. *PLoS One*. **9**, e89597.(2014)
Mortality, all causes	POI → increased risk of death from all causes	Menopause <40 years vs 45-49 years, HR=2.10 (95% CI 1.07-4.11)	Cox PH adjusted for age, SBP, serum total cholesterol level, HDL cholesterol, level, history of diabetes mellitus, BMI, smoking habits, alcohol drinking habits, marital status, study area, and type of menopause	Jichi Medical School Cohort Study, Japan	4,683	1903-1959 (aged 36-89 years in 1992-1995)	Amagai, Ishikawa et al. Age at menopause and mortality in Japan: the Jichi Medical School Cohort Study. *J Epidemiol*. **16**, 161-166.(2006)
Mortality, all causes	POI → increased risk of death from all causes	Menopause <40 years vs 45-49 years, risk of death increased, HR=1.32 (95% CI 1.05-1.66, p=0.02)	Cox PH adjusted for age, alcohol consumption, education, age at first birth, self-cognitive health level, chronic disease, marital partner, parity, age at menarche, oral contraceptive use and hypertension	Kangwha Cohort, South Korea	2,658	Aged 55+ years 1985-2001	Hong, Yi et al. Age at menopause and cause-specific mortality in South Korean women: Kangwha Cohort Study. *Maturitas*. **56**, 411-419.(2007)
Mortality, cancer	Early menopause → increased risk cancer mortality	Compared with menopause at 50+ years, increased cancer mortality for menopause at 40-44 years, RR=2.34 (95% CI 1.20-4.58)	Age-stratified and Poisson regression adjusted for age, duration of follow-up, race, education, smoking, and use of HRT	National Health and Examination Survey (NHANES) Epidemiologic Follow-up Study, USA	3,191	Aged 50-86 years	Cooper and Sandler. Age at natural menopause and mortality. *Ann Epidemiol*. **8**, 229-235.(1998)
Mortality, cancer	Early menopause → increased risk cancer mortality	Menopause at 40–44 years, RR = 2.34 (95% CI 1.20–4.58)	Poisson regression controlled for age, years follow-up, race, education, smoking, HRT use	NHANES	3,191	1897-1950 (25-74 years in 1971-1975)	Cooper and Sandler. Age at natural menopause and mortality. *Ann Epidemiol*. **8**, 229-235.(1998)

Outcome	Effect on outcome	Effect of variable	Analysis type	Study	n	Birth year of cohort	Reference
Mortality, cancer	POI → increased risk mortality from cancer	Menopause <40 years vs 45-49 years, risk of death from cancer, HR=2.01 (95% CI 1.06-3.82, p=0.03)	Cox PH adjusted for age, alcohol consumption, education, age at first birth, self-cognitive health level, chronic disease, marital partner, parity, age at menarche, oral contraceptive use and hypertension	Kangwha Cohort, South Korea	2,658	Aged 55+ years 1985-2001	Hong, Yi et al. Age at menopause and cause-specific mortality in South Korean women: Kangwha Cohort Study. *Maturitas*. **56**, 411-419.(2007)
Mortality, cancer	POI → increased risk mortality from cancer	Note: 73% of POF cases were natural. For mortality from cancer, HR=1.37 (95% CI 1.04-1.80). Similar when HRT users excluded.	Logistic regression adjusted for age, income, occupation, BMI, waist hip ratio, smoking, nulliparity, age at menarche, type of menopause and HRT use.	Shanghai Women's Health Study, China	1003 cases POI	1926-1960 (40-70 years 1996-2000)	Wu, Cai et al. Impact of premature ovarian failure on mortality and morbidity among Chinese women. *PLoS One*. **9**, e89597.(2014)
Mortality, coronary heart disease	Earlier menopause → increased risk of death from ischaemic heart disease	Per year delayed menopause, for mortality from ischaemic heart disease HR=0.90 (95% CI 0.80-1.00)	Cox PH adjusted for age, type of menopause, parity, age at first birth, BMI, smoking, hypertensions, diabetes, previous CVD	Diagnostisch Onderzoek Mammacarcinoom, Utrecht, The Netherlands	12,134	Aged 48-68 in 1974-1977	Ossewaarde, Bots et al. Age at menopause, cause-specific mortality and total life expectancy. *Epidemiology*. **16**, 556-562.(2005)
Mortality, coronary heart disease	Early menopause → higher risk of death from coronary heart disease	All cause mortality from coronary heart disease higher in menopause 40-44 years vs 50-54 years (RR=1.09 (95% CI 1.00-1.18))	20 years follow up. Excluded women ever taking HRT or ever smoking. Cox PH adjusted for age, race, marital status, BMI, age at menarche, parity, educational level, alcohol comsumption, oral contraceptive use, exercise.	Cancer Prevention Study II	68,154	Aged 30+ years in 1982.	Mondul, Rodriguez et al. Age at natural menopause and cause-specific mortality. *Am J Epidemiol*. **162**, 1089-1097.(2005)
Mortality, coronary heart disease	Early menopause → increased ischaemic heart disease mortality	Menopause age in categories of 35–40 years, 41–44 years, 45–48 years, 49–51 years (reference), 52–55 years, and 56–60 years. P=0.05 for linear trend in ischaemic heart disease mortality, decreasing with menopause age (adjusted model).	Cox PH adjusted for diabetes, hypertension, parity, age at first birth, and physical activity in leisure.	Adventist Health Study, California, USA	5,279	pre-1951 (25 and older in 1976 at recruitment)	Jacobsen, Knutsen et al. Age at natural menopause and total mortality and mortality from ischemic heart disease: the Adventist Health Study. *J Clin Epidemiol*. **52**, 303-307.(1999)
Mortality, CVD	Earlier menopause → increased risk of death from CVD	Per year delayed menopause, for mortality from CVD HR=0.90 (95% CI 0.84-0.97)	Cox PH adjusted for age, type of menopause, parity, age at first birth, BMI, smoking, hypertensions, diabetes, previous CVD	Diagnostisch Onderzoek Mammacarcinoom, Utrecht, The Netherlands	12,134	Aged 48-68 in 1974-1977	Ossewaarde, Bots et al. Age at menopause, cause-specific mortality and total life expectancy. *Epidemiology*. **16**, 556-562.(2005)
Mortality, CVD	Earlier menopause → increased risk of death from CVD	Menopause >51 years vs <45 years associated with reduced CVD mortality, HR=0.82 (95% CI 0.69-0.98)	Natural and surgical menopause combined (77% natural). Cox PH adjusted for HRT, hypertension, BMI, SES.	Nijmegen, The Netherlands	9,450	Aged 35-65 in 1975	de Kleijn, van der Schouw et al. Endogenous estrogen exposure and cardiovascular mortality risk in postmenopausal women. *Am J Epidemiol*. **155**, 339-345.(2002)

Outcome	Effect on outcome	Effect of variable	Analysis type	Study	n	Birth year of cohort	Reference
Mortality, CVD	Earlier menopause → increased risk of death from CVD	CVD mortality decreased by 2% per year increase in menopause age, HR=0.982 (95% CI 0.968-0.996)	Cox PH adjusted for age.	Utrecht, The Netherlands	12,115	Aged 50-65 years	van der Schouw, van der Graaf et al. Age at menopause as a risk factor for cardiovascular mortality. *Lancet*. **347**, 714-718.(1996)
Mortality, CVD	POI → increased risk of death from CVD	Menopause <40 years vs 45-49 years, risk of death from CVD, HR=1.53 (95% CI 1.00-2.39, p=0.04)	Cox PH adjusted for age, alcohol consumption, education, age at first birth, self-cognitive health level, chronic disease, marital partner, parity, age at menarche, oral contraceptive use and hypertension	Kangwha Cohort, South Korea	2,658	Aged 55+ years 1985-2001	Hong, Yi et al. Age at menopause and cause-specific mortality in South Korean women: Kangwha Cohort Study. *Maturitas*. **56**, 411-419.(2007)
Mortality, external causes	Early menopause → higher risk of death from external causes	All cause mortality from external causes higher in menopause 40-44 years vs 50-54 years (RR=1.56 (95% CI 1.21-2.02))	20 years follow up. Excluded women ever taking HRT or ever smoking. Cox PH adjusted for age, race, marital status, BMI, age at menarche, parity, educational level, alcohol comsumption, oral contraceptive use, exercise.	Cancer Prevention Study II	68,154	Aged 30+ years in 1982.	Mondul, Rodriguez et al. Age at natural menopause and cause-specific mortality. *Am J Epidemiol*. **162**, 1089-1097.(2005)
Mortality, genitourinary disease	Early menopause → higher risk of death from genitourinary disease	All cause mortality from genitourinary disease higher in menopause 40-44 years vs 50-54 years (RR=1.39 (95% CI 1.07-1.82))	20 years follow up. Excluded women ever taking HRT or ever smoking. Cox PH adjusted for age, race, marital status, BMI, age at menarche, parity, educational level, alcohol comsumption, oral contraceptive use, exercise.	Cancer Prevention Study II	68,154	Aged 30+ years in 1982.	Mondul, Rodriguez et al. Age at natural menopause and cause-specific mortality. *Am J Epidemiol*. **162**, 1089-1097.(2005)
Mortality, ovarian or uterine cancer	Later menopause → increased risk of death from ovarian or uterine cancer	Per year delayed menopause, for mortality from ovarian or uterine cancer HR=1.07 (95% CI 1.01-1.12)	Cox PH adjusted for age, type of menopause, parity, age at first birth, BMI, smoking, hypertensions, diabetes, previous CVD	Diagnostisch Onderzoek Mammacarcinoom, Utrecht, The Netherlands	12,134	Aged 48-68 in 1974-1977	Ossewaarde, Bots et al. Age at menopause, cause-specific mortality and total life expectancy. *Epidemiology*. **16**, 556-562.(2005)
Mortality, respiratory disease	Early menopause → higher risk of death from respiratory disease	All cause mortality from respiratory disease higher in menopause 40-44 years vs 50-54 years (RR=1.19 (95% CI 1.02-1.39))	20 years follow up. Excluded women ever taking HRT or ever smoking. Cox PH adjusted for age, race, marital status, BMI, age at menarche, parity, educational level, alcohol comsumption, oral contraceptive use, exercise.	Cancer Prevention Study II	68,154	Aged 30+ years in 1982.	Mondul, Rodriguez et al. Age at natural menopause and cause-specific mortality. *Am J Epidemiol*. **162**, 1089-1097.(2005)
Walking speed	Early menopause → slower walking in old age	When aged 60+ years, walking speed 0.05 meters/second (95% CI 0.01-0.10) faster for menopause ≥ 55 years vs <45 years.	Women aged 60+ years. Adjusted for age, race/ethnicity, height, weight, educational attainment, smoking status, number of children, and use of oestrogen therapy	National Health and Nutrition Examination Survey III, USA	1,765	Before 1934 (aged 60+ years in 1988-1994)	Tom, Cooper et al. Menopausal characteristics and physical functioning in older adulthood in the National Health and Nutrition Examination Survey III. *Menopause*. **19**, 283-289.(2012)

Outcome	Effect on outcome	Effect of variable	Analysis type	Study	n	Birth year of cohort	Reference
Cancer, upper GI tract	Post-menopausal → increased risk of oesophageal carcinoma	Menopausal women were at increased risk of oesophageal carcinoma RR=1.47 (95% CI 1.07-2.03, P = 0.018), oesophageal squamous cell carcinoma RR=1.66 (95% CI 1.12-2.48, P = 0.012)	Meta-analysis pooled RR from 16 studies				Wang, Zhang et al. Hormonal and reproductive factors and risk of esophageal cancer in women: a meta-analysis. *Dis Esophagus*.(2015)

Appendix 2. Epidemiological risk factors associated with age at menopause.

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Alcohol	Higher alcohol consumption → later menopause	For 14+ units per week between 25-49 years vs none, HR=0.89 (95% CI 0.86,0.92). p<0.001 for trend	Competing risks Cox PH adjusted for age, smoking status, parity, BMI at 40 years.	Breakthrough Generations Study, UK	50,678	Aged 40-98 in 2003-2011	Morris, Jones et al. Body mass index, exercise, and other lifestyle factors in relation to age at natural menopause: analyses from the breakthrough generations study. *Am J Epidemiol*. **175**, 998-1005.(2012)
Alcohol	Alcohol → later menopause	Never drink HR =1.10 (95% CI 1.04,1.16) vs low drinker	Multivariate Cox PH, adjusted for age, population, education, marital status, smoking, BMI, physical activity, alcohol consumption, supplementation with vitamins and minerals, hormonal contraceptives, HRT.	Health, Alcohol and Psychosocial factors in Eastern Europe (HAPIEE), Russia, Poland, Czech Republic	12,676	Aged 45-69 in 2002-2005	Stepaniak, Szafraniec et al. Age at natural menopause in three central and eastern European urban populations: the HAPIEE study. *Maturitas*. **75**, 87-93.(2013)
Alcohol	Alcohol → later menopause	Drinker p=0.001, log-rank vs non-drinker. Not significant. in Cox PH.	Log-rank. Cox PH adjusted.	Australian Twin Registry	5,961	Aged 17-88 in 1980-82, or aged 50+ in 1993-1995	Do, Treloar et al. Predictive factors of age at menopause in a large Australian twin study. *Hum Biol*. **70**, 1073-1091.(1998)
Alcohol	Alcohol → later menopause	<1 drink per week vs none in last year, OR=0.7 (95% CI 0.5,1.0), 1+ drinks per week vs none in last year, OR=0.7 (95% CI 0.5,1.0)	Odds of being post-menopausal vs premenopausal, age adjusted	US 1999 National Health Interview Survey (NHIS)	3,307	1945-1959	Brett and Cooper. Associations with menopause and menopausal transition in a nationally representative US sample. *Maturitas*. **45**, 89 - 97.(2003)
Alcohol	Increase in alcohol consumption → later menopause	Increased alcohol consumption (in 10 years follow-up) vs no change, HR=0.90 (95% CI 0.83,0.98)	Cox PH adjusted for race/ethnicity, baseline smoking, time-varying smoking, health baseline, educational level, use of oral contraceptives at baseline, alcohol at baseline, alcohol change since baseline, current employment, physical activity, percentile of baseline weight. 10 years follow-up	Study of Women's Health Across the Nation (SWAN), USA	3,302	Aged 40-55 in 1995-1997	Gold, Crawford et al. Factors related to age at natural menopause: longitudinal analyses from SWAN. *Am J Epidemiol*. **178**, 70-83.(2013)
Alcohol	Higher alcohol consumption → later menopause	Compared with no drinking, 1.3 year later (95% CI 0.2,2.3) for 1+ days per week, 2.2 year later (95% CI 0.5,3.9) for drinking 5-7 days per week	Parametric logistic survival analysis. Multi-variate model including pregnancy outcome, alcohol, caffeine and smoking	Case-control study of spontaneous abortion, New York, USA	494	1933-1942	Kinney, Kline et al. Alcohol, caffeine and smoking in relation to age at menopause. *Maturitas*. **54**, 27-38.(2006)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
BMI	Lower BMI → earlier menopause	BMI <20 as reference; BMI 20-24.9 HR=0.94 (95% CI 0.92,0.97), BMI 25-29.9 HR=0.92 (95% CI 0.89,0.94), BMI≥30 HR=0.92 (95% CI 0.89,0.95). p<0.0001 for test of trend.	Age at menopause collected as categories (<45, 45-49, 50-54, ≥55). Mulitvariable Cox PH adjusted for ethnicity, smoking, menarche age, parity, BMI	Multiethnic Cohort Study, USA (non-Latina Whites, Japanese Americans, African Americans, Native Hawai'ians, and Latinas).	95,704	Aged 45-74 years in 1993-1996	Henderson, Bernstein et al. Predictors of the timing of natural menopause in the Multiethnic Cohort Study. *Am J Epidemiol*. **167**, 1287-1294.(2008)
BMI	Lower BMI → earlier menopause	BMI at 40 years, BMI 18.5-24.9 as reference. BMI<18.5 HR=1.13 (95% CI 1.02,1.25), BMI≥30 HR=0.86 (95% CI 0.82, 0.91); p<0.001 for trend	Competing risks Cox PH adjusted for age, smoking status, parity.	Breakthrough Generations Study, UK	50,678	Aged 40-98 in 2003-2011	Morris, Jones et al. Body mass index, exercise, and other lifestyle factors in relation to age at natural menopause: analyses from the breakthrough generations study. *Am J Epidemiol*. **175**, 998-1005.(2012)
BMI	Lower BMI → earlier menopause	BMI at age 20 ≥21.4 vs BMI<18.0, beta=0.22 p<0.01	Multi-variable linear regression, including age, education, occupation, income, marital status, current employment, energy intake, weight gain between age 20 and 50 years, cigarette smoking (never/ever), and leisure-time physical activity patterns in adolescence and adulthood.	Shanghai Women's Health Study	33,054	Aged 40-70 years in 1997-2000	Dorjgochoo, Kallianpur et al. Dietary and lifestyle predictors of age at natural menopause and reproductive span in the Shanghai Women's Health Study. *Menopause*. **15**, 924-933.(2008)
BMI	Lower BMI → earlier menopause	BMI <24.9 51.1 years vs BMI 25.0-29.9 51.2 years vs BMI ≥30 51.3 years. p<0.05	Chi-squared. ANCOVA adjusted for age, education, BMI, smoking, age at menarche, menstrual cycle, parity and oral contraceptive use.	Patients at hospitals in Italy	31,834	Aged ≥55 1997-2003	Parazzini. Determinants of age at menopause in women attending menopause clinics in Italy. *Maturitas*. **56**, 280-287.(2007)
BMI	Lower BMI → earlier menopause	Increasing BMI RR=0.937 (95% CI 0.920,0.955)	Multiple logistic regression, adjusted for age, age at menarche, number of pregnancies, BMI, past history of infertility, past history of endometriosis and smoking before menopause. Relative risk (RR) - age adjusted OR.	Japan Nurses' Health Study (JNHS)	24,153	Aged 40-59 years 2001-2007	Yasui, Hayashi et al. Association of endometriosis-related infertility with age at menopause. *Maturitas*. **69**, 279-283.(2011)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
BMI	Lower BMI → earlier menopause	BMI ≥25 HR=0.694 (95% CI 0.597,0.807) vs BMI<18.5	Multivariate Cox PH, endpoints menopause, early menopause (<45 years), premature ovarian failure (<40 years). Adjusted for age at menarche, number of deliveries, current BMI, cycle regularity, unilateral oophorectomy, oral contraceptives, ever smoker before menopause and birth year decade.	Japan Nurses' Health Study (JNHS)	24,152	Aged 40-59 years 2001-2007	Yasui, Hayashi et al. Factors associated with premature ovarian failure, early menopause and earlier onset of menopause in Japanese women. *Maturitas*. **72**, 249-255.(2012)
BMI	Lower BMI → earlier menopause	BMI 25-29.9 HR=0.94 (95% CI 0.89,0.97), BMI 30+ HR 0.90 (95% CI 0.73,0.94) vs BMI <18.5. 4 categories, p=0.012 for trend.	Cox PH, multivariate model with education, monthly income, BMI, age at menarche, parity, smoking	Women attending health screening in Jiangsu Province of China	20,275	Aged 40-65 in 2010-2011	Li, Wu et al. Factors associated with the age of natural menopause and menopausal symptoms in Chinese women. *Maturitas*. **73**, 354-360.(2012)
BMI	Lower BMI → earlier menopause	BMI ≥30, HR=0.78 (95% CI 0.67,0.90) vs BMI 20-24	Multivariate Cox PH including parity, age at menarche, oral contraceptive use, unilateral oophorectomy, smoking status, education, vigorous physical activity, and BMI	Black Women's Health Study, USA	17,070	Aged 35-55 in 1995	Palmer, Rosenberg et al. Onset of natural menopause in African American women. *Am J Public Health*. **93**, 299-306.(2003)
BMI	Lower BMI → earlier menopause	BMI≤27 vs BMI>27, p<0.001 for association.	Log-rank test, unadjusted	DOM-3 breast screening programme in Utrecht, The Netherlands	8,701	1932-1941	de Vries, den Tonkelaar et al. Oral contraceptive use in relation to age at menopause in the DOM cohort. *Hum Reprod*. **16**, 1657-1662.(2001)
BMI	High BMI → earlier menopause	BMI ≥30 vs BMI 18.5-24.9, HR=1.318 (95% CI 1.022,1.698)	Cox PH, adjusted for country	European Respiratory Health Survey (Spain, France, Belgium, Switzerland, UK, Norway, Sweden, Iceland, and Estonia) and the Swiss Air Pollution and Lung Disease in Adults Cohort	5,288	Aged 30-60 1998-2002	Dratva, Gomez Real et al. Is age at menopause increasing across Europe? Results on age at menopause and determinants from two population-based studies. *Menopause*. **16**, 385-394.(2009)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
BMI	Lower BMI → earlier menopause	1.7 years difference between highest and lowest quartiles of BMI, p<0.05	Comparison of menopause age in Q1, Q2+Q2, Q4 BMI at age 40/41 years	Nagasaki, Japan 1958 onwards	1,136	Women in Nagasaki exposed to atomic bomb in 1945	Akahoshi, Soda et al. The effects of body mass index on age at menopause. *Int J Obes Relat Metab Disord*. **26**, 961-968.(2002)
BMI, change	Greatest increase in BMI from age 25 to menopause → later menopause	Highest quartile of BMI gain, HR = 0.832 (95% CI 0.765,0.905).	Cox PH. Weight change 25 years to menopause.	Isparta Menopause and Health Study, Turkey (hospital based study)	1,106	Not known	Aydin. Determinants of age at natural menopause in the Isparta Menopause and Health Study: premenopausal body mass index gain rate and episodic weight loss. *Menopause*. **17**, 494-505.(2010)
BMI, change	Episodic weight loss >5kg → later menopause	Episodic weight loss of more than 5kg HR=0.433 (95% CI 0.344,0.546)	Cox PH. Weight change 25 years to menopause.	Isparta Menopause and Health Study, Turkey (hospital based study)	1,106	Not known	Aydin. Determinants of age at natural menopause in the Isparta Menopause and Health Study: premenopausal body mass index gain rate and episodic weight loss. *Menopause*. **17**, 494-505.(2010)
Childhood, birth weight	Extreme birthweight → earlier menopause	Birthweight 3.0-3.49 kg as reference, <2.5kg OR=1.91 (95% CI 1.08,3.38), ≥4.0kg OR=1.81 (95% CI 1.11,2.97) .	Logistic regression, odds menopause by 44-45 years, adjusted for SES, smoking status, oral contraceptives	1958 British Birth Cohort, UK	2,900	1958	Tom, Cooper et al. Fetal environment and early age at natural menopause in a British birth cohort study. *Hum Reprod*. **25**, 791-798.(2010)
Childhood, birth weight	Heavier birthweight of twin → earlier menopause	Twin with menopause <35 years was significantnificantly heavier at birth than twin with menopause >56 years (p=0.028) (2774 g vs 2185 g)	ANOVA, linear regression, correlations	Australian National Health and Medical Council Twin Registry	646	17-88 in 1980-1982; >50 years in 1993-1995	Treloar, Sadrzadeh et al. Birth weight and age at menopause in Australian female twin pairs: exploration of the fetal origin hypothesis. *Hum Reprod*. **15**, 55-59.(2000)
Childhood, breastfed	Breastfeeding → later menopause	p=0.05	Cox PH, menopause before/after 50 years considered separately	Medical Research Council National Survey of Health and Development (1946 Birth Cohort)	1,583	1946	Mishra, Hardy et al. Are the effects of risk factors for timing of menopause modified by age? Results from a British birth cohort study. *Menopause*. **14**, 717-724.(2007)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Childhood, breastfed	Breastfeeding for longer as child → later menopause	Breastfeeding 7+ months vs never HR=0.74 (95% CI 0.57,0.97). P=0.01 for test for trend (≤3 months vs 4-6 months vs 7+ months) in full childhood growth model.	Cox PH, adjusted for parity, smoking, BMI, SES, unilateral oophorectomy. Fulll childhood growth model included birthweight, weight at 2 years, breastfed, and crowding at 2 years.	Medical Research Council National Survey of Health and Development (1946 Birth Cohort)	1,572	1946	Hardy and Kuh. Does early growth influence timing of the menopause? Evidence from a British birth cohort. *Hum Reprod*. **17**, 2474-2479.(2002)
Childhood, cognitive ability	Lower cognitive score as child → earlier menopause	For 1 s.d. increase in cognitive score, HR=0.8 (95% CI 0.72,0.90) for Aberdeen followed to 49 years, HR=0.87 (95% CI 0.79,0.95) for 1946 Birth Cohort followed to 49 years, HR=0.87 (95% CI 0.79,0.95) for 1946 Birth Cohort followed to 53 years	Cox PH, unadjusted	1946 British Birth Cohort, Aberdeen cohort study, UK	4,815	1946, 1950-56	Kuh, Butterworth et al. Childhood cognitive ability and age at menopause: evidence from two cohort studies. *Menopause*. **12**, 475-482.(2005)
Childhood, foetal growth	Faster foetal growth → earlier menopause	Fastest growing quartile vs second fastest growing quartile, OR=1.80 (95% CI 1.16,2.81)	Logistic regression, odds menopause by 44-45 years, adjusted for SES, smoking status, oral contraceptives	1958 British Birth Cohort, UK	2,900	1958	Tom, Cooper et al. Fetal environment and early age at natural menopause in a British birth cohort study. *Hum Reprod*. **25**, 791-798.(2010)
Childhood, food deprivation	Exposure to famine at 2-6 years → earlier menopause	Severe food deprivation at age 2-6, -1.83 years (95% CI -3.03,-0.63)	Differences in mean menopause age in not, moderately and severly exposed. Adjusted for smoking, parity, SES, BMI, age at menarche, year of birth.	Population based cohort, Utrecht, The Netherlands	9,471	Aged 40-73 years in 1983-1986	Elias, van Noord et al. Caloric restriction reduces age at menopause: the effect of the 1944-1945 Dutch famine. *Menopause*. **10**, 399-405.(2003)
Childhood, gestational food deprivation	Gestational exspoure to famine → earlier menopause	Any gestational exposure to famine vs none, HR=1.32 (95% CI 1.05,1.66)	Competing risks Cox PH, adjusted for smoking, birthweight	Birth cohort, The Netherlands	558	1945-1946	Yarde, Broekmans et al. Prenatal famine, birthweight, reproductive performance and age at menopause: the Dutch hunger winter families study. *Hum Reprod*. **28**, 3328-3336.(2013)
Childhood, maternal smoking	Exposure to maternal smoke in pregnancy → earlier menopause in never smokers	Maternal smoking vs none, HR=1.21 (95% CI 1.02,1.43)	Cox PH adjusted for BMI, education, age at menarche, marital status, pregnancy and birth histories, unilateral oophorectomy, HRT, and oral contraceptive use	National Cooperative Diethylstilbestrol Adenosis (DESAD) Project, USA	4,025	1939-1968	Strohsnitter, Hatch et al. The association between in utero cigarette smoke exposure and age at menopause. *Am J Epidemiol*. **167**, 727-733.(2008)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Childhood, parental divorce	Parental divorce during childhood → earlier menopause	Parental divorce in childhood, HR=6.5 (95% CI 2.0,21.3) for menopause under 50 years; HR=2.5 (95% CI 1.5,4.2) for menopause over 50 years	Cox PH, menopause before/after 50 years considered separately	Medical Research Council National Survey of Health and Development (1946 Birth Cohort)	1,583	1946	Mishra, Hardy et al. Are the effects of risk factors for timing of menopause modified by age? Results from a British birth cohort study. *Menopause*. **14**, 717-724.(2007)
Childhood, parental divorce	Parental divorce as young child → earlier menopause	Parental divorce before age of 5 years HR(unadjusted)=2.14 (95% CI 1.33,3.42)	Cox PH, adjusted (i) for adult lifestyle (smoking, BMI, parity, marital status) (ii) psychological health (iii) childhood factors (cognitive score at age 8 years, breast feeding).	Medical Research Council National Survey of Health and Development (1946 Birth Cohort)	1,515	1946	Hardy and Kuh. Social and environmental conditions across the life course and age at menopause in a British birth cohort study. *Bjog*. **112**, 346-354.(2005)
Childhood, SES	Lower SES as child → earlier menopause	Manual vs non-manual class in childhood, -0.61 years (95% CI -1.22,0.00)	Multiple linear regression, adjusting for age, age menarche, nulliparity, every use of oral contraception, use of hormone replacement therapy, smoking, BMI (quadratic term included) and all indicators of adult socio-economic position	British Women's Heart and Health Study	3,513	Aged 60-79 years in 1999-2001	Lawlor, Ebrahim et al. The association of socio-economic position across the life course and age at menopause: the British Women's Heart and Health Study. *Bjog*. **110**, 1078-1087.(2003)
Childhood, SES	No bathroom in house as child → earlier menopause	No bathroom in house when child vs bathroom, -0.40 years (95% CI -0.78,-0.02)	Multiple linear regression, adjusting for age, age menarche, nulliparity, every use of oral contraception, use of hormone replacement therapy, smoking, BMI (quadratic term included) and all indicators of adult socio-economic position	British Women's Heart and Health Study	3,513	Aged 60-79 years in 1999-2001	Lawlor, Ebrahim et al. The association of socio-economic position across the life course and age at menopause: the British Women's Heart and Health Study. *Bjog*. **110**, 1078-1087.(2003)
Childhood, SES	Crowding at 2 years → earlier menopause	Crowding in house at age 2 (2+ persons per room), HR=1.30 (95% CI 1.02,1.65). Not significant. in full childhood growth model.	Cox PH, adjusted for parity, smoking, BMI, SES, unilateral oophorectomy	Medical Research Council National Survey of Health and Development (1946 Birth Cohort)	1,572	1946	Hardy and Kuh. Does early growth influence timing of the menopause? Evidence from a British birth cohort. *Hum Reprod*. **17**, 2474-2479.(2002)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Childhood, SES	Lower SES as child → earlier menopause	Father always a manual worker (at 2, 11 and 15 years) vs never, HR(unadjusted)=1.4 (95% CI 1.13,1.73). HR(adjusted)=1.13 (95% CI 1.04,1.22) in model of childhood social class, adult housing tenure, and hardship as adult.	Cox PH, adjusted (i) for adult lifestyle (smoking, BMI, parity, marital status) (ii) psychological health (iii) childhood factors (cognitive score at age 8 years, breast feeding).	Medical Research Council National Survey of Health and Development (1946 Birth Cohort)	1,515	1946	Hardy and Kuh. Social and environmental conditions across the life course and age at menopause in a British birth cohort study. *Bjog*. **112**, 346-354.(2005)
Childhood, siblings	Fewer siblings → later menopause	4.5 siblings for early menopause vs 4.2 for late, p=0.033	Chi-squared, or t-test. Not adjusted. Early menopause (<49 years) vs. late menopause (≥53 years)	Doorlopend Onderz oek Morbidi teit/Mortaliteit [DOM], The Netherlands	3,756	1911-1925	van Noord, Dubas et al. Age at natural menopause in a population-based screening cohort: the role of menarche, fecundity, and lifestyle factors. *Fertil Steril*. **68**, 95-102.(1997)
Childhood, weight	Heavier at 2 years → later menopause	Child in heaviest group at 2 years vs lightest, HR=0.41 (95% CI 0.16,1.01) for menopause under 50 year, HR=0.65 (95% CI 0.42,1.00) for menopause after 50 years	Cox PH, menopause before/after 50 years considered separately	Medical Research Council National Survey of Health and Development (1946 Birth Cohort)	1,583	1946	Mishra, Hardy et al. Are the effects of risk factors for timing of menopause modified by age? Results from a British birth cohort study. *Menopause*. **14**, 717-724.(2007)
Childhood, weight	Higher weight at 2 years old → later menopause	For highest vs lowest weight at 2 years, HR=0.75, p=0.04 (test for trend)	Cox PH, adjusted for parity, smoking, BMI, SES, unilateral oophorectomy, in fulll childhood growth model including birthweight, weight at 2 years, breastfed, and crowding at 2 years.	Medical Research Council National Survey of Health and Development (1946 Birth Cohort)	1,572	1946	Hardy and Kuh. Does early growth influence timing of the menopause? Evidence from a British birth cohort. *Hum Reprod*. **17**, 2474-2479.(2002)
Childhood, weight	Lower weight at age 1 year → earlier menopause	Lower weight at 1 yr and earlier menopause, p=0.03. Adjusted for weight, smoking.	Multiple linear regression, t-tests, Fisher's exact test	Women born in Hertfordshire and Sheffield, UK	990	1923-1930 in Hertforshire, 1952-53 in Sheffield.	Cresswell, Egger et al. Is the age of menopause determined in-utero? *Early Human Development*. **49**, 143-148.(1997)
Cognitive ability	Increasing cognitive ability → later menopause	For menopause before/after 50, increasing cognitive ability associated with increasing age at menopause, p=0.009	Cox PH, menopause before/after 50 years considered separately	Medical Research Council National Survey of Health and Development (1946 Birth Cohort)	1,583	1946	Mishra, Hardy et al. Are the effects of risk factors for timing of menopause modified by age? Results from a British birth cohort study. *Menopause*. **14**, 717-724.(2007)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Diet, calories per day	Higher calories → later menopause	>2005.1 kcal/day vs ≤1281.5, beta=0.39, p<0.01	Multi-variable linear regression, including age, education, occupation, income, marital status, current employment, energy intake, weight gain between age 20 and 50 years, cigarette smoking (never/ever), and leisure-time physical activity patterns in adolescence and adulthood.	Shanghai Women's Health Study	33,054	Aged 40-70 years in 1997-2000	Dorjgochoo, Kallianpur et al. Dietary and lifestyle predictors of age at natural menopause and reproductive span in the Shanghai Women's Health Study. *Menopause*. **15**, 924-933.(2008)
Diet, carbohydrates	Higher carbohydrate intake → later menopause	>346.8 g/day vs ≤223.9 g/day, beta=0.24, p=0.04	Multi-variable linear regression, including age, education, occupation, income, marital status, current employment, energy intake, weight gain between age 20 and 50 years, cigarette smoking (never/ever), and leisure-time physical activity patterns in adolescence and adulthood.	Shanghai Women's Health Study	33,054	Aged 40-70 years in 1997-2000	Dorjgochoo, Kallianpur et al. Dietary and lifestyle predictors of age at natural menopause and reproductive span in the Shanghai Women's Health Study. *Menopause*. **15**, 924-933.(2008)
Diet, carbohydrates	Higher carbohydrate intake → earlier menopause	Carbohydrates highest quartile vs lowest, HR=1.47 (95% CI 1.07,2.02) (all quartiles significant.)	Cox PH adjusted for age, education, age at menarche, oral contraceptive use, HRT-use, parity, BMI, time of breast feeding, age at first full term pregnancy, smoking habit, ethanol intake and leisure time physical activity, total energy intake	European prospective investigation into cancer and nutrition (EPIC) - Heidelberg, Germany	4,807	Aged 35-65 in 1994-1998	Nagel, Altenburg et al. Reproductive and dietary determinants of the age at menopause in EPIC-Heidelberg. *Maturitas*. **52**, 337-347.(2005)
Diet, cereals	Higher cereal product intake → earlier menopause	Cereal product intake, highest quartile vs lowest HR=1.25 (95% CI 1.02-1.53) (Q3 and Q4 significant.)	Cox PH adjusted for age, education, age at menarche, oral contraceptive use, HRT-use, parity, BMI, time of breast feeding, age at first full term pregnancy, smoking habit, ethanol intake and leisure time physical activity, total energy intake	European prospective investigation into cancer and nutrition (EPIC) - Heidelberg, Germany	4,807	Aged 35-65 in 1994-1998	Nagel, Altenburg et al. Reproductive and dietary determinants of the age at menopause in EPIC-Heidelberg. *Maturitas*. **52**, 337-347.(2005)
Diet, fat	Low fat dairy products → later menopause (in women premenopausal at <51 years)	In premenopausal women aged <51 years in 1980: >3 servings of low-fat dairy per day vs 0.1–1 servings/day, HR =0.86 (95% CI 0.77,0.96); >6 servings/wk skim milk vs. 0–1 servings/month HR= 0.93 (95% CI 0.89,0.97).	Cox PH, multivariate models - age at menarche, age at first birth, parity, exercise, BMI, oral contrceptive use, smoking, marital status, total energy, red meat consumption, egg consumption	US Nurses Health Study (NHS). Follow-up 1980-2000	46,059	Aged 30-55 in 1976	Carwile, Willett et al. Consumption of low-fat dairy products may delay natural menopause. *J Nutr*. **143**, 1642-1650.(2013)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Diet, fat	Higher fat intake → later menopause	Total fat, quartile 3 vs lowest, HR=0.78 (95% 0.65,0.97) (Q3 significant only)	Cox PH adjusted for age, education, age at menarche, oral contraceptive use, HRT-use, parity, BMI, time of breast feeding, age at first full term pregnancy, smoking habit, ethanol intake and leisure time physical activity, total energy intake	European prospective investigation into cancer and nutrition (EPIC) - Heidelberg, Germany	4,807	Aged 35-65 in 1994-1998	Nagel, Altenburg et al. Reproductive and dietary determinants of the age at menopause in EPIC-Heidelberg. *Maturitas*. **52**, 337-347.(2005)
Diet, fibre	Higher fibre intake → earlier menopause	Fibre intake, highest quartile vs lowest, HR=1.30 (95% CI 1.03,1.65) (Q2 and Q4 significant)	Cox PH adjusted for age, education, age at menarche, oral contraceptive use, HRT-use, parity, BMI, time of breast feeding, age at first full term pregnancy, smoking habit, ethanol intake and leisure time physical activity, total energy intake	European prospective investigation into cancer and nutrition (EPIC) - Heidelberg, Germany	4,807	Aged 35-65 in 1994-1998	Nagel, Altenburg et al. Reproductive and dietary determinants of the age at menopause in EPIC-Heidelberg. *Maturitas*. **52**, 337-347.(2005)
Diet, food deprivation	Exposure to famine → earlier menopause	Severe exposure 0.36 years earlier (95% CI -0.6,-0.11)	Differences in mean menopause age in not, moderately and severly exposed. Adjusted for smoking, parity, SES, BMI, age at menarche, year of birth.	Population based cohort, Utrecht, The Netherlands	9,471	Aged 40-73 years in 1983-1986	Elias, van Noord et al. Caloric restriction reduces age at menopause: the effect of the 1944-1945 Dutch famine. *Menopause*. **10**, 399-405.(2003)
Diet, fruit	Higher fruit intake → later menopause	Fruit >383.2 g/day vs ≤73.3 g/day, beta=0.13, p=0.04	Multi-variable linear regression, including age, education, occupation, income, marital status, current employment, energy intake, weight gain between age 20 and 50 years, cigarette smoking (never/ever), and leisure-time physical activity patterns in adolescence and adulthood.	Shanghai Women's Health Study	33,054	Aged 40-70 years in 1997-2000	Dorjgochoo, Kallianpur et al. Dietary and lifestyle predictors of age at natural menopause and reproductive span in the Shanghai Women's Health Study. *Menopause*. **15**, 924-933.(2008)
Diet, meat	Higher meat intake → later menopause	Meat intake, highest quartile vs lowest, HR=0.75 (95% CI 0.61,0.93) (Q4 significant only)	Cox PH adjusted for age, education, age at menarche, oral contraceptive use, HRT-use, parity, BMI, time of breast feeding, age at first full term pregnancy, smoking habit, ethanol intake and leisure time physical activity, total energy intake	European prospective investigation into cancer and nutrition (EPIC) - Heidelberg, Germany	4,807	Aged 35-65 in 1994-1998	Nagel, Altenburg et al. Reproductive and dietary determinants of the age at menopause in EPIC-Heidelberg. *Maturitas*. **52**, 337-347.(2005)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Diet, protein	Higher protein intake → later menopause	Protein >82.7g/day vs ≤45.7 g/day, beta=0.24, p=0.02	Multi-variable linear regression, including age, education, occupation, income, marital status, current employment, energy intake, weight gain between age 20 and 50 years, cigarette smoking (never/ever), and leisure-time physical activity patterns in adolescence and adulthood.	Shanghai Women's Health Study	33,054	Aged 40-70 years in 1997-2000	Dorjgochoo, Kallianpur et al. Dietary and lifestyle predictors of age at natural menopause and reproductive span in the Shanghai Women's Health Study. *Menopause*. **15**, 924-933.(2008)
Diet, vegetables	Vegetarian → earlier menopause	Vegetarian by 40 years vs not, HR=1.12 (95% CI 1.06,1.20)	Competing risks Cox PH adjusted for age, smoking status, parity, BMI at 40 years.	Breakthrough Generations Study, UK	50,678	Aged 40-98 in 2003-2011	Morris, Jones et al. Body mass index, exercise, and other lifestyle factors in relation to age at natural menopause: analyses from the breakthrough generations study. *Am J Epidemiol*. **175**, 998-1005.(2012)
Diet, vegetables	Higher vegetable intake → earlier menopause	Vegetable intake, Q3 vs lowest, HR=1.32 (95% CI 1.09,1.60) (Q3 significant only)	Cox PH adjusted for age, education, age at menarche, oral contraceptive use, HRT-use, parity, BMI, time of breast feeding, age at first full term pregnancy, smoking habit, ethanol intake and leisure time physical activity, total energy intake	European prospective investigation into cancer and nutrition (EPIC) - Heidelberg, Germany	4,807	Aged 35-65 in 1994-1998	Nagel, Altenburg et al. Reproductive and dietary determinants of the age at menopause in EPIC-Heidelberg. *Maturitas*. **52**, 337-347.(2005)
Diet, vitamins/minerals	Supplementation with vitamins/minerals → later menopause	Supplements vs none, HR=0.96 (95% CI 0.92,1.00)	Multivariate Cox PH, adjusted for age, population, education, marital status, smoking, BMI, physical activity, alcohol consumption, supplementation with vitamins and minerals, hormonal contraceptives, HRT.	Health, Alcohol and Psychosocial factors in Eastern Europe (HAPIEE), Russia, Poland, Czech Republic	12,676	Aged 45-69 in 2002-2005	Stepaniak, Szafraniec et al. Age at natural menopause in three central and eastern European urban populations: the HAPIEE study. *Maturitas*. **75**, 87-93.(2013)
Education	Less education → earlier menopause	Low education 51.1 years, high school/university degree 51.3 years, p<0.05	Chi-squared. ANCOVA adjusted for age, education, BMI, smoking, age at menarche, menstrual cycle, parity and oral contraceptive use.	Patients at hospitals in Italy	31,834	Aged ≥55 in 1997-2003	Parazzini. Determinants of age at menopause in women attending menopause clinics in Italy. *Maturitas*. **56**, 280-287.(2007)
Education	Less education → earlier menopause	Graduated college 48.6 years vs 47.4 years for less than high school (p<0.0001)	Multiple linear regression of geographical region, race, age, parity, smoking, region, income, education, physical activity, and history of CVD	REasons for Geographic And Racial Differences in Stroke and Myocardial Infarction (REGARDS), USA	22,484	Aged >=45 in 2003-2007)	McKnight, Wellons et al. Racial and regional differences in age at menopause in the United States: findings from the REasons for Geographic And Racial Differences in Stroke (REGARDS) study. *Am J Obstet Gynecol*. **205**, 353.e351-358.(2011)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Education	Less education → earlier menopause	High school or less vs bachelor's degree, HR=1.15 (95% CI 1.06,1.26)	Multivariate Cox PH including age, race/ethnicity, education, childhood family income, smoking status in 40s	Sister Study, USA (US and Puerto Rican women)	22,165	Aged 35-74 years in 2003-2007	Steiner, D'Aloisio et al. Association of intrauterine and early-life exposures with age at menopause in the sister study. *American Journal of Epidemiology*. **172**, 140-148.(2010)
Education	Less education → earlier menopause	College/university vs elementary school, HR=0.86 (95% CI 0.77,0.96). p=0.016 for trend.	Cox PH, multivariate model with education, monthly income, BMI, age at menarche, parity, smoking	Women attending health screening in Jiangsu Province of China	20,275	Aged 40-65 in 2010-2011	Li, Wu et al. Factors associated with the age of natural menopause and menopausal symptoms in Chinese women. *Maturitas*. **73**, 354-360.(2012)
Education	Less education → earlier menopause	High school HR=1.48 (95% CI 1.26,1.73), some college HR=1.29 (95% CI 1.10,1.51) vs graduate/professional school.	Cox PH, adjusted for smoking, education, marital status, heart disease, parity, race/ethnicity, employment, oral contraceptives	Study of Women's Health Across the Nation (SWAN), USA	14,620	Aged 40-55 in 1995-1997	Gold, Bromberger et al. Factors associated with age at natural menopause in a multiethnic sample of midlife women. *American Journal of Epidemiology*. **153**, 865 - 874.(2001)
Education	Less education → earlier menopause	University vs secondary or lower, HR=0.85 (95% CI 0.82,0.90).	Multivariate Cox PH, adjusted for age, population, education, marital status, smoking, BMI, physical activity, alcohol consumption, supplementation with vitamins and minerals, hormonal contraceptives, HRT.	Health, Alcohol and Psychosocial factors in Eastern Europe (HAPIEE), Russia, Poland, Czech Republic	12,676	Aged 45-69 in 2002-2005	Stepaniak, Szafraniec et al. Age at natural menopause in three central and eastern European urban populations: the HAPIEE study. *Maturitas*. **75**, 87-93.(2013)
Education	Less education → earlier menopause	Secondary HR=0.85 (95% CI 0.78,0.91), high/academic HR=0.72 (95% CI 0.62,0.84), vs primary/apprenticeship	Cox PH, adjusted for educational level, age at menarche, menstrual cycle length, oral contraceptives, live births, smoking status, and self-reported health status.	WOMID, Poland	7,183	Aged 35-65 in 2000-2004	Kaczmarek. The timing of natural menopause in Poland and associated factors. *Maturitas*. **57**, 139-153.(2007)
Education	Less education → earlier menopause	University degree, HR=0.71 (95% CI 0.58,0.87) vs school. P=0.0001 for log-rank.	Log-rank. Cox PH adjusted.	Australian Twin Registry	5,961	Aged 17-88 in 1980-82, or aged 50+ in 1993-1995	Do, Treloar et al. Predictive factors of age at menopause in a large Australian twin study. *Hum Biol*. **70**, 1073-1091.(1998)
Education	Less education → earlier menopause	Low education HR=1.16 (95% CI 1.07,1.26)	Cox PH	Women from 7 cities in Latin America and the Caribbean	4,056	Aged 60-79 years	Velez, Alvarado et al. Life course socioeconomic adversity and age at natural menopause in women from Latin America and the Caribbean. *Menopause*. **17**, 552-559.(2010)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Education	Less education → earlier menopause	Degree vs less than high school, OR=0.4 (95% CI 0.3,0.6)	Odds of being post-menopausal vs premenopausal, age adjusted	US 1999 National Health Interview Survey (NHIS)	3,307	1945-1959	Brett and Cooper. Associations with menopause and menopausal transition in a nationally representative US sample. *Maturitas*. **45**, 89 - 97.(2003)
Education	Less education → earlier menopause	College degree vs high school or less, HR=0.77 (95% CI 0.66,0.90)	Cox PH adjusted for race/ethnicity, baseline smoking, time-varying smoking, health baseline, educational level, use of oral contraceptives at baseline, alcohol at baseline, alcohol change since baseline, current employment, physical activity, percentile of baseline weight. 10 years follow-up	Study of Women's Health Across the Nation (SWAN), USA	3,302	Aged 40-55 in 1995-1997	Gold, Crawford et al. Factors related to age at natural menopause: longitudinal analyses from SWAN. *Am J Epidemiol*. **178**, 70-83.(2013)
Education	Less education → earlier menopause	Percentage of menopause <40 years, highest with lowest education. p=0.001 for association	Chi-squared test against age at menopause in 4 categories (<40, 40-44, 45-49, ≥50)	National Health and Nutrition Examination Survey (NHANES), USA	3,191	Aged 25-74 years in 1971-1975	Cooper and Sandler. Age at natural menopause and mortality. *Ann Epidemiol*. **8**, 229-235.(1998)
Education	Less education → earlier menopause	For >12 years education vs <8 years, OR= 0.50 (95% CI 0.34,0.72)	Logistic regression, case was menopause at <45 years, adjusted for smoking, alcohol, coffee and education.	Oslo Health Study, Norway	2,123	1940-41 (collected at age 59-60 years)	Mikkelsen, Graff-Iversen et al. Early menopause, association with tobacco smoking, coffee consumption and other lifestyle factors: a cross-sectional study. *BMC Public Health*. **7**, 149.(2007)
Education	Less education → earlier menopause	Education for ≤8 years as reference; OR=0.82 (0.68,0.97) for 9-10 years; OR=0.75 (0.59,0.96) for ≥11 years; p=0.004	Cox PH multivariate model including smoking, hormone use, BMI, age at first full-term pregnancy	National Finnish Register	1,505	Aged 45-64 in 1989	Luoto, Kaprio et al. Age at natural menopause and sociodemographic status in Finland. *Am J Epidemiol*. **139**, 64-76.(1994)
Education	Less education → earlier menopause	For ≤12 years education vs 12+ years, beta (hazard) -0.16 years, p=0.045	Multivariate stepwise PH regression including geographic area, marital status, income, education, history of irregular menstrual periods, parity, and ever use of oral contraceptives. Comparison of medians, Mantel-Cox test for differences.	Breast Cancer Detection Demonstration Project (BCDDP), USA	1,423	Aged 22-62 years in 1973-1977	Stanford, Hartge et al. Factors influencing the age at natural menopause. *J Chronic Dis*. **40**, 995-1002.(1987)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Education	Less education → earlier menopause	No education vs tertiary education, OR=2.9 (95% CI 1.3,6.1) .	Logistic regression (odds of being menopausal) adjusted for age, education, occupation, marital status, history of dysmenorrhea, age at first pregnancy, number of pregnancies, number of deliveries, history of hypertension, oral contraceptive use and HRT use.	Cluster sample of women aged 40-60 years in Ibadan, Nigeria	1,189	Aged 40-60 years in 2006-2007	OlaOlorun and Lawoyin. Age at menopause and factors associated with attainment of menopause in an urban community in Ibadan, Nigeria. *Climacteric*. **12**, 352-363.(2009)
Education	Less education → earlier menopause	Secondary education vs university education, HR=1.77 (95% CI 1.07,2.93)	Cox PH adjusted for education, parity, marital status, BMI, smoking, alcohol consumption, age at menarche and oral contraceptive use for Spanish and Latin-American models. For Latin-American models, also place of birth and whether or not women had experienced menopause in their country of origin	Decisions at Menopause Study (2002–2003), Madrid, Spain	484	Aged 45-55 in 2002-2003; Aged 45-55 in 2010-2011	Perez-Alcala, Sievert et al. Cross cultural analysis of factors associated with age at natural menopause among Latin-American immigrants to Madrid and their Spanish neighbors. *Am J Hum Biol*. **25**, 780-788.(2013)
Environmental exposure, combustion by-product	Higher 1,2,3,4,6,7,8-heptachlorodibenzofuran → earlier menopause	Effect of top 10% exposure vs rest, -1.77 ($p<0.05$)	Linear regression adjusted for time of interview, race/ethnicity, BMI, current smoking status.	National Health and Nutrition Examination Survey (NHANES), USA	1,442	Mean age 60.8 years in 1998-2008	Grindler, Allsworth et al. Persistent organic pollutants and early menopause in u.s. Women. *PLoS One*. **10**, e0116057.(2015)
Environmental exposure, coolants	Polychlorinated biphenyl congeners (PCBs) → earlier menopause	Dose-response effect of -0.31 to -0.71 per decile ($p<0.05$)	Linear regression adjusted for time of interview, race/ethnicity, BMI, current smoking status.	National Health and Nutrition Examination Survey (NHANES), USA	1,442	Mean age 60.8 years in 1998-2008	Grindler, Allsworth et al. Persistent organic pollutants and early menopause in u.s. Women. *PLoS One*. **10**, e0116057.(2015)
Environmental exposure, lead	Exposure to lead → earlier menopause	Highest tibia lead content vs lowest, -1.21 years (95% CI -2.08,-0.35)	Linear regression adjusted for age at menarche, year of birth, substudy group, age at lead measurement, oral contraceptive use, parity, pack-years smoking.	Nurses Health Study	449	Aged 30-55 in 1976	Eum, Weisskopf et al. Cumulative lead exposure and age at menopause in the Nurses' Health Study cohort. *Environ Health Perspect*. **122**, 229-234.(2014)
Environmental exposure, pesticide	Higher organophosphate pesticide → earlier menopause	Dose-response effect of -0.12 to -0.34 per decile ($p<0.05$)	Linear regression adjusted for time of interview, race/ethnicity, BMI, current smoking status.	National Health and Nutrition Examination Survey (NHANES), USA	1,442	Mean age 60.8 years in 1998-2008	Grindler, Allsworth et al. Persistent organic pollutants and early menopause in u.s. Women. *PLoS One*. **10**, e0116057.(2015)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Environmental exposure, plasticiser	Higher mono-(-2-ethyl-5-hydroxylhexy)/ mono-(-2-ethyl-5-oxohexyl) pthalate→ earlier menopause	Dose-response effect of -0.17 to -0.22 per decile (p<0.05)	Linear regression adjusted for time of interview, race/ethnicity, BMI, current smoking status.	National Health and Nutrition Examination Survey (NHANES), USA	1,442	Mean age 60.8 years in 1998-2008	Grindler, Allsworth et al. Persistent organic pollutants and early menopause in u.s. Women. *PLoS One*. **10**, e0116057.(2015)
Environmental exposure, pre-natal oestrogen	Diethylstilbestrol exposure in utero → earlier menopause	Exposure to diethylstilbestrol vs non-exposed, HR=1.45 (95% CI 1.27,1.65)	Multivariate Cox PH including age, race/ethnicity, education, childhood family income, smoking status in 40s	Sister Study, USA (US and Puerto Rican women)	22,165	Aged 35-74 years in 2003-2007	Steiner, D'Aloisio et al. Association of intrauterine and early-life exposures with age at menopause in the sister study. *American Journal of Epidemiology*. **172**, 140-148.(2010)
Environmental exposure, pre-natal oestrogen	Pre-natal exposure to diethylstilbesterol → earlier menopause	Diethylstilbestrol exposed vs non-exposed, HR=1.49 (95% CI 1.28,1.74)	Cox PH, adjusted for birthweight, age at menarche, marital status,smoking, parity, contraception, unilatral oophorectomy, HRT	NCI Diethylstilbestrol Follow-up Study	6,039	1950s, 1960s	Hatch, Troisi et al. Age at natural menopause in women exposed to diethylstilbestrol in utero. *Am J Epidemiol*. **164**, 682-688.(2006)
Environmental exposure, radiation	Radiation exposure → earlier menopause	Median age at menopause 0.3 years younger in females exposed to radiation of 1 Gy	Poisson regression adjusted for city, birth year, parity, smoking, age at menarche, age, radiation dose, age at radiation exposure	Life Span Study of people exposed to atomic bombs in Nagasaki or Hiroshima, Japan	21,259	1894-1945	Sakata, Shimizu et al. Effect of radiation on age at menopause among atomic bomb survivors. *Radiat Res*. **176**, 787-795.(2011)
Exercise	Higher exercise → later menopause	Regular strenuous exercise aged 30-49 years vs none, HR=0.96 (0.93,0.98) .	Competing risks Cox PH adjusted for age, smoking status, parity, BMI at 40 years.	Breakthrough Generations Study, UK	50,678	Aged 40-98 in 2003-2011	Morris, Jones et al. Body mass index, exercise, and other lifestyle factors in relation to age at natural menopause: analyses from the breakthrough generations study. *Am J Epidemiol*. **175**, 998-1005.(2012)
Exercise	Higher exercise → later menopause	Moderate-high activity vs low activity, beta=1.35, p=0.048. Exercise 4+ hrs/wk/year vs 0 hrs/wk/year, beta=0.47, p<0.01. Moderate-high adolescent and adult activity vs low adult and adolescent activity, beta=0.23, p<0.01 .	Multi-variable linear regression, including age, education, occupation, income, marital status, current employment, energy intake, weight gain between age 20 and 50 years, cigarette smoking (never/ever), and leisure-time physical activity patterns in adolescence and adulthood.	Shanghai Women's Health Study	33,054	Aged 40-70 years in 1997-2000	Dorjgochoo, Kallianpur et al. Dietary and lifestyle predictors of age at natural menopause and reproductive span in the Shanghai Women's Health Study. *Menopause*. **15**, 924-933.(2008)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Exercise	No exercise → earlier menopause	No exercise 47.8 years, 1-3 times per week 48.2 years (p=0.0007), 4+ times per week 48.1 years (p=0.01)	Multiple linear regression of geographical region, race,age, parity, smoking, region, income, education, physical activity, and history of CVD	REasons for Geographic And Racial Differences in Stroke and Myocardial Infarction (REGARDS), USA	22,484	Aged >=45 in 2003-2007)	McKnight, Wellons et al. Racial and regional differences in age at menopause in the United States: findings from the REasons for Geographic And Racial Differences in Stroke (REGARDS) study. *Am J Obstet Gynecol*. **205**, 353.e351-358.(2011)
Exercise	No exercise → earlier menopause	No physical activity vs 1-5 hrs a week, HR=1.06 (95% CI 1.00,1.12)	Multivariate Cox PH, adjusted for age, population, education, marital status, smoking, BMI, physical activity, alcohol consumption, supplementation with vitamins and minerals, hormonal contraceptives, HRT.	Health, Alcohol and Psychosocial factors in Eastern Europe (HAPIEE), Russia, Poland, Czech Republic	12,676	Aged 45-69 in 2002-2005	Stepaniak, Szafraniec et al. Age at natural menopause in three central and eastern European urban populations: the HAPIEE study. *Maturitas*. **75**, 87-93.(2013)
Exercise	No exercise → earlier menopause	Women aged 40-49 years at baseline, low activity HR=0.70 (95% CI 0.53,0.92), high activity HR=0.72 (95% CI 0.55,0.96), vs no activity	Cox PH, adjusted for adjusting for age at menarche, parity, use of oral contraceptives prior to the 6 months preceding participation in HUNT 2, symptoms of depression, smoking status, and education.	Health Survey in Nord-Trøndelag (HUNT 2 1995-97, HUNT3 2006-2008)	8,454	Aged ≥20 years in 1995-1997	Gudmundsdottir, Flanders et al. Physical activity and age at menopause: the Nord-Trondelag population-based health study. *Climacteric*. **16**, 78-87.(2013)
Exercise	No exercise → earlier menopause	Women aged 40-49 years at baseline, <1 hr per week HR=0.67 (95% CI 0.51,0.86) to ≥3 hrs/week HR=0.77 (95% CI 0.60,0.99) vs no activity	Cox PH, adjusted for adjusting for age at menarche, parity, use of oral contraceptives prior to the 6 months preceding participation in HUNT 2, symptoms of depression, smoking status, and education.	Health Survey in Nord-Trøndelag (HUNT 2 1995-97, HUNT3 2006-2008)	8,454	Aged ≥20 years in 1995-1997	Gudmundsdottir, Flanders et al. Physical activity and age at menopause: the Nord-Trondelag population-based health study. *Climacteric*. **16**, 78-87.(2013)
Exercise	Low exercise → earlier menopause	Low activity vs medium, HR=1.367 (95% CI 1.118,1.672)	Cox PH, adjusted for country	European Respiratory Health Survey (Spain, France, Belgium, Switzerland, UK, Norway, Sweden, Iceland, and Estonia) and the Swiss Air Pollution and Lung Disease in Adults Cohort	5,288	Aged 30-60 1998-2002	Dratva, Gomez Real et al. Is age at menopause increasing across Europe? Results on age at menopause and determinants from two population-based studies. *Menopause*. **16**, 385-394.(2009)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Exercise	Higher exercise → earlier menopause	More physical activity during follow-up vs no change, HR = 1.07 (95% CI 1.02,1.12)	Cox PH adjusted for race/ethnicity, baseline smoking, time-varying smoking, health baseline, educational level, use of oral contraceptives at baseline, alcohol at baseline, alcohol change since baseline, current employment, physical activity, percentile of baseline weight. 10 years follow-up	Study of Women's Health Across the Nation (SWAN), USA	3,302	Aged 40-55 in 1995-1997	Gold, Crawford et al. Factors related to age at natural menopause: longitudinal analyses from SWAN. *Am J Epidemiol*. **178**, 70-83.(2013)
Exercise	Medium exercise → earlier menopause	Medium vs low activity, HR (menopause <52 years) =1.26 (95% CI 1.031,1.542)	Cox PH analysis in women with menopause <52 years and 52+ years, adjusted for smoking status, parity, age at menarche, oral contraceptive, education, BMI, physical activity, language region	SAPALDIA Swiss cohort study on Air Pollution and Lung Diseases in Adults (SAPALDIA)	3,119	Aged 18-60 years in 1992	Dratva, Zemp et al. Variability of reproductive history across the Swiss SAPALDIA cohort--patterns and main determinants. *Ann Hum Biol*. **34**, 437-453.(2007)
Geographical region, Europe	Czech Republic or Russia (compared with Poland) → earlier menopause	Polish towns as reference, Czech towns HR=1.11 (95% CI 1.05,1.17), Russian town HR=1.48 (95% CI 1.40,1.57)	Multivariate Cox PH, adjusted for age, population, education, marital status, smoking, BMI, physical activity, alcohol consumption, supplementation with vitamins and minerals, hormonal contraceptives, HRT.	Health, Alcohol and Psychosocial factors in Eastern Europe (HAPIEE), Russia, Poland, Czech Republic	12,676	Aged 45-69 in 2002-2005	Stepaniak, Szafraniec et al. Age at natural menopause in three central and eastern European urban populations: the HAPIEE study. *Maturitas*. **75**, 87-93.(2013)
Geographical region, USA	Southern USA → earlier menopause	South USA as reference (47.5 years), Northest 48.4 years (p<0.0001), Midwest 48.2 years (p=0.0001), West 48.0 years (p=0.02)	Multiple linear regression of geographical region, race,age, parity, smoking, region, income, education, physical activity, and history of CVD	REasons for Geographic And Racial Differences in Stroke and Myocardial Infarction (REGARDS), USA	22,484	Aged >=45 in 2003-2007)	McKnight, Wellons et al. Racial and regional differences in age at menopause in the United States: findings from the REasons for Geographic And Racial Differences in Stroke (REGARDS) study. *Am J Obstet Gynecol*. **205**, 353.e351-358.(2011)
Health, blood pressure	Increasing blood pressure premenopause → earlier menopause	Per 10mm Hg change in blood pressure, increase -7.38 years (95% CI -10.78,-3.98), decrease not significant	Linear regression adjusted for smoking	Framingham Heart Study cohort	695	Aged 29-62 years in 1948	Kok, van Asselt et al. Heart disease risk determines menopausal age rather than the reverse. *J Am Coll Cardiol*. **47**, 1976-1983.(2006)
Health, cholesterol change premenopause	Increasing cholesterol premenopause → earlier menopause	Per 20 mg/dl increase premenopause vs none, -2.60 years (95% CI -4.06, -1.14)	Linear regression adjusted for smoking	Framingham Heart Study cohort	695	Aged 29-62 years in 1948	Kok, van Asselt et al. Heart disease risk determines menopausal age rather than the reverse. *J Am Coll Cardiol*. **47**, 1976-1983.(2006)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Health, cholesterol change premenopause	Decreasing cholesterol level premenopause → later menopause	Per 20 mg/dl cholesterol decrease premenopause vs none, 4.16 years (95% CI 0.08,8.24)	Linear regression adjusted for smoking	Framingham Heart Study cohort	695	Aged 29-62 years in 1948	Kok, van Asselt et al. Heart disease risk determines menopausal age rather than the reverse. *J Am Coll Cardiol*. **47**, 1976-1983.(2006)
Health, cholesterol premenopause	Higher premenopausal cholesterol → earlier menopause	Premenopausal cholesterol per 20 mg/dl, -0.14 years (95% CI -0.26,-0.00)	Linear regression adjusted for smoking	Framingham Heart Study cohort	695	Aged 29-62 years in 1948	Kok, van Asselt et al. Heart disease risk determines menopausal age rather than the reverse. *J Am Coll Cardiol*. **47**, 1976-1983.(2006)
Health, heart disease	Heart disease → earlier menopause	Heart disease 47.9 years vs 48.2 years for no heart disease (p=0.04)	Multiple linear regression of geographical region, race,age, parity, smoking, region, income, education, physical activity, and history of CVD	REasons for Geographic And Racial Differences in Stroke and Myocardial Infarction (REGARDS), USA	22,484	Aged >=45 in 2003-2007)	McKnight, Wellons et al. Racial and regional differences in age at menopause in the United States: findings from the REasons for Geographic And Racial Differences in Stroke (REGARDS) study. *Am J Obstet Gynecol*. **205**, 353.e351-358.(2011)
Health, heart disease	Heart disease → earlier menopause	Heart disease vs no heart disease, HR=1.36 (95% CI 1.08,1.71)	Cox PH, adjusted for smoking, education, marital status, heart disease, parity, race/ethnicity, employment, oral contraceptives	Study of Women's Health Across the Nation (SWAN), USA	14,620	Aged 40-55 in 1995-1997	Gold, Bromberger et al. Factors associated with age at natural menopause in a multiethnic sample of midlife women. *American Journal of Epidemiology*. **153**, 865 - 874.(2001)
Health, heart disease risk score	Increased Framingham risk score → earlier menopause	Per 1% increase in 10 year risk for coronary heart disease, -1.8 years (95% CI -2.72 ,-0.92).	Linear regression adjusted for smoking	Framingham Heart Study cohort	695	Aged 29-62 years in 1948	Kok, van Asselt et al. Heart disease risk determines menopausal age rather than the reverse. *J Am Coll Cardiol*. **47**, 1976-1983.(2006)
Health, premenopausal T2 diabates	T2 diabetes → earlier menopause	T2 diabetes vs none, HR=1.640 (95% CI 1.141,2.356)	Cox PH. Adjusted for episodic weight loss of more than 5kg.	Isparta Menopause and Health Study, Turkey (hospital based study)	1,106	Not known	Aydin. Determinants of age at natural menopause in the Isparta Menopause and Health Study: premenopausal body mass index gain rate and episodic weight loss. *Menopause*. **17**, 494-505.(2010)
Health, self-reported	Poorer health → earlier menopause	Good health HR=0.84 (95% CI 0.66,0.95), fair HR=0.89 (95% CI 0.80,0.99) vs poor.	Cox PH, adjusted for educational level, age at menarche, menstrual cycle length, oral contraceptives, live births, smoking status, and self-reported health status.	WOMID, Poland	7,183	Aged 35-65 in 2000-2004	Kaczmarek. The timing of natural menopause in Poland and associated factors. *Maturitas*. **57**, 139-153.(2007)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Health, self-reported	Poorer health → earlier menopause	Excellent self-reported health vs poor, HR=1.11 (95% CI 1.04,1.19)	Cox PH adjusted for race/ethnicity, baseline smoking, time-varying smoking, health baseline, educational level, use of oral contraceptives at baseline, alcohol at baseline, alcohol change since baseline, current employment, physical activity, percentile of baseline weight. 10 years follow-up	Study of Women's Health Across the Nation (SWAN), USA	3,302	Aged 40-55 in 1995-1997	Gold, Crawford et al. Factors related to age at natural menopause: longitudinal analyses from SWAN. *Am J Epidemiol*. **178**, 70-83.(2013)
Height, adult	Short height → earlier menopause	Height <158 cm HR=1.04 (95% CI 1.01,1.07), 158-161 cm HR=1.03 (95% CI 1.00,1.07), vs 162-165 cm. Test for trend not significant.	Competing risks Cox PH adjusted for age, smoking status, parity, BMI at 40 years.	Breakthrough Generations Study, UK	50,678	Aged 40-98 in 2003-2011	Morris, Jones et al. Body mass index, exercise, and other lifestyle factors in relation to age at natural menopause: analyses from the breakthrough generations study. *Am J Epidemiol*. **175**, 998-1005.(2012)
Height, adult	Short height → earlier menopause	Per 1 s.d. increase in height, 0.20 years (95% CI 0.01,0.40)	Multiple linear regression, adjusting for age, age menarche, nulliparity, every use of oral contraception, use of hormone replacement therapy, smoking, weight, and all indicators of childhood and adult socio-economic position	British Women's Heart and Health Study	3,513	Aged 60-79 years in 1999-2001	Lawlor, Ebrahim et al. The association of socio-economic position across the life course and age at menopause: the British Women's Heart and Health Study. *Bjog*. **110**, 1078-1087.(2003)
Marital status	Married → later menopause	For married vs never married, 0.4 years (p=0.02)	ANCOVA - adjusted	Women visiting GPs in Italy in 1997	16,916	Aged 44-60 in 1997	Amigoni, Morelli et al. Cross-sectional study of determinants of menopausal age and hormone replacement therapy use in Italian women. *Climacteric*. **3**, 25-32.(2000)
Marital status	Separated/widowed/divorced → earlier menopause	Separated/widowed/divorced vs married/living as married, HR=1.27 (95% CI 1.14,1.41)	Cox PH, adjusted for smoking, education, marital status, heart disease, parity, race/ethnicity, employment, oral contraceptives	Study of Women's Health Across the Nation (SWAN), USA	14,620	Aged 40-55 in 1995-1997	Gold, Bromberger et al. Factors associated with age at natural menopause in a multiethnic sample of midlife women. *American Journal of Epidemiology*. **153**, 865 - 874.(2001)
Marital status	Married → later menopause	Not married vs married, OR=1.6 (95% CI 1.0,2.5)	Logistic regression (odds of being menopausal) adjusted for age, education, occupation, marital status, history of dysmenorrhea, age at first pregnancy, number of pregnancies, number of deliveries, history of hypertension, oral contraceptive use and HRT use.	Cluster sample of women aged 40-60 years in Ibadan, Nigeria	1,189	Aged 40-60 years in 2006-2007	OlaOlorun and Lawoyin. Age at menopause and factors associated with attainment of menopause in an urban community in Ibadan, Nigeria. *Climacteric*. **12**, 352-363.(2009)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Marital status	Married → later menopause	Currently married has lower % of menopausal than all other women, chi-squared p<0.05	Chi- squared tests of % menopausal by age group (45-49 years vs 50-54 years)	Survey in London, UK	736	Aged 45-54 years in 1965	McKinlay, Jefferys et al. An investigation of the age at menopause. *J Biosoc Sci*. **4**, 161-173.(1972)
Maternal age at menopause	Increasing age of mother at menopause → later menopause	Decreasing HR with increasing age of mother at menopause in menopause before/after 50, p<0.0001	Cox PH, menopause before/after 50 years considered separately	Medical Research Council National Survey of Health and Development (1946 Birth Cohort)	1,583	1946	Mishra, Hardy et al. Are the effects of risk factors for timing of menopause modified by age? Results from a British birth cohort study. *Menopause*. **14**, 717-724.(2007)
Race/ethnicity	Latina → earlier menopause	US born Latina, HR=1.10 (95% CI 1.07,1.14), non-Us born Latina HR=1.25 (95% CI 1.21,1.30), vs white	Age at menopause collected as categories (<45, 45-49, 50-54, ≥55). Mulitvariable Cox PH adjusted for ethnicity, smoking, menarche age, parity, BMI	Multiethnic Cohort Study, USA (non-Latina Whites, Japanese Americans, African Americans, Native Hawai'ians, and Latinas).	95,704	Aged 45-74 years in 1993-1996	Henderson, Bernstein et al. Predictors of the timing of natural menopause in the Multiethnic Cohort Study. *Am J Epidemiol*. **167**, 1287-1294.(2008)
Race/ethnicity	Japanese → later menopause	Japanese American HR=0.93 (95% CI 0.90,0.95) vs white	Age at menopause collected as categories (<45, 45-49, 50-54, ≥55). Mulitvariable Cox PH adjusted for ethnicity, smoking, menarche age, parity, BMI	Multiethnic Cohort Study, USA (non-Latina Whites, Japanese Americans, African Americans, Native Hawai'ians, and Latinas).	95,704	Aged 45-74 years in 1993-1996	Henderson, Bernstein et al. Predictors of the timing of natural menopause in the Multiethnic Cohort Study. *Am J Epidemiol*. **167**, 1287-1294.(2008)
Race/ethnicity	Japanese → later menopause	Japanese vs Caucasian, HR=0.74 (95% CI 0.55,1.00)	Cox PH, adjusted for smoking, education, marital status, heart disease, parity, race/ethnicity, employment, oral contraceptives	Study of Women's Health Across the Nation (SWAN), USA	14,620	Aged 40-55 in 1995-1997	Gold, Bromberger et al. Factors associated with age at natural menopause in a multiethnic sample of midlife women. *American Journal of Epidemiology*. **153**, 865 - 874.(2001)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Race/ethnicity	Non white ethnicity → earlier menopause	Higher % of non-white ethnicity with menopause <40 years, p=0.001.	Chi-squared test against age at menopause in 4 categories (<40, 40-44, 45-49, ≥50)	National Health and Nutrition Examination Survey (NHANES), USA	3,191	Aged 25-74 years in 1971-1975	Cooper and Sandler. Age at natural menopause and mortality. *Ann Epidemiol*. **8**, 229-235.(1998)
Race/ethnicity	Latin-American immigrant → earlier menopause	-1.5 years for Latin-American immigrants vs Spanish (p<0.001)	Cox PH adjusted for education, parity, marital status, BMI, smoking, alcohol consumption, age at menarche and oral contraceptive use for Spanish and Latin-American models. For Latin-American models, also place of birth and whether or not women had experienced menopause in their country of origin	Decisions at Menopause Study (2002–2003), Madrid, Spain	484	Aged 45-55 in 2002-2003; Aged 45-55 in 2010-2011	Perez-Alcala, Sievert et al. Cross cultural analysis of factors associated with age at natural menopause among Latin-American immigrants to Madrid and their Spanish neighbors. *Am J Hum Biol*. **25**, 780-788.(2013)
Refugee	Refugee → earlier menopause	Refugee vs non-refugee, HR=1.33 (95% CI 1.02,1.75)	Cox PH, adjusted	Women living in Tuzla, Bosnia and Herzegovina during war in 1992-1995 and up until interview	331	Aged 39-75 years in 2009-2011	Balic, Rizvanovic et al. Age at natural menopause in refugee and domicile women who lived in Tuzla Canton in Bosnia and Herzegovina during and after the war. *Menopause*. **21**, 721-725.(2014)
Religion	Jewish → later menopause	Jewish vs Catholic, HR=0.81 (95% CI 0.72,0.91)	Cox PH, mutivariate model of age, parity, religion, BMI, cigarettes per day.	New York University Women Study, USA	4,694	Mean age 42.8 years in 1985-1991	Kato, Toniolo et al. Prospective study of factors influencing the onset of natural menopause.[see comment]. *Journal of Clinical Epidemiology*. **51**, 1271 - 1276.(1998)
Reproductive, age at birth of first daughter	Later age at birth when had daughter → later menopause	Increasing age at birth of first daughter, beta=0.17 years, p=0.017	Linear regression, women with children, adjusted for birth year of mother	Survey of women in Poland	465	Aged 44-98 years	Galbarczyk and Jasienska. Timing of natural menopause covaries with timing of birth of a first daughter: evidence for a mother-daughter evolutionary contract? *Homo*. **64**, 228-232.(2013)
Reproductive, age at first birth	Younger age at first live birth → later menopause	First birth at 30+ years vs <20 years, beta=-0.49, p<0.01	Multi-variable linear regression, including age, education, occupation, income, marital status, current employment, energy intake, weight gain between age 20 and 50 years, cigarette smoking (never/ever), and leisure-time physical activity patterns in adolescence and adulthood.	Shanghai Women's Health Study	33,054	Aged 40-70 years in 1997-2000	Dorjgochoo, Kallianpur et al. Dietary and lifestyle predictors of age at natural menopause and reproductive span in the Shanghai Women's Health Study. *Menopause*. **15**, 924-933.(2008)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Reproductive, age at first birth	Younger age at first birth → earlier menopause	Higher % of births <18 with menopause <40 years, p=0.002	Chi-squared test against age at menopause in 4 categories (<40, 40-44, 45-49, ≥50)	National Health and Nutrition Examination Survey (NHANES), USA	3,191	Aged 25-74 years in 1971-1975	Cooper and Sandler. Age at natural menopause and mortality. *Ann Epidemiol*. **8**, 229-235.(1998)
Reproductive, age at first pregnancy	Younger at first pregnancy → earlier menopause	First pregnancy at 32+ years vs <20 years, HR=0.69 (95% CI 0.51,0.94) .	Cox PH adjusted for age.	European prospective investigation into cancer and nutrition (EPIC) - Heidelberg, Germany	4,807	Aged 35-65 in 1994-1998	Nagel, Altenburg et al. Reproductive and dietary determinants of the age at menopause in EPIC-Heidelberg. *Maturitas*. **52**, 337-347.(2005)
Reproductive, age at first pregnancy	Younger at first pregnancy → earlier menopause	First pregnancy at 10-17 years as reference, 25+ years OR=0.2 (95% CI 0.09,0.6), 18-24 years OR=0.3 (95% CI 0.1,0.7)	Logistic regression (odds of being menopausal) adjusted for age, education, occupation, marital status, history of dysmenorrhea, age at first pregnancy, number of pregnancies, number of deliveries, history of hypertension, oral contraceptive use and HRT use.	Cluster sample of women aged 40-60 years in Ibadan, Nigeria	1,189	Aged 40-60 years in 2006-2007	OlaOlorun and Lawoyin. Age at menopause and factors associated with attainment of menopause in an urban community in Ibadan, Nigeria. *Climacteric*. **12**, 352-363.(2009)
Reproductive, ageatlastbirth	Younger age at last live birth → earlier menopause	For 35+ years vs <25 years, beta=-0.17, p<0.01	Multi-variable linear regression, including age, education, occupation, income, marital status, current employment, energy intake, weight gain between age 20 and 50 years, cigarette smoking (never/ever), and leisure-time physical activity patterns in adolescence and adulthood.	Shanghai Women's Health Study	33,054	Aged 40-70 years in 1997-2000	Dorjgochoo, Kallianpur et al. Dietary and lifestyle predictors of age at natural menopause and reproductive span in the Shanghai Women's Health Study. *Menopause*. **15**, 924-933.(2008)
Reproductive, ageatlastpregnancy	Younger age at last pregnancy → earlier menopause	46 years, p=0.001	ANOVA, not adjusted	Women in city of Shiraz, Iran	948	Menopausal in 2000	Ayatollahi, Ghaem et al. Menstrual-reproductive factors and age at natural menopause in Iran. *Int J Gynaecol Obstet*. **80**, 311-313.(2003)
Reproductive, birth control	Intrauterine device → later menopause	Intrauterine device vs no, beta=0.18, p<0.01	Multi-variable linear regression, including age, education, occupation, income, marital status, current employment, energy intake, weight gain between age 20 and 50 years, cigarette smoking (never/ever), and leisure-time physical activity patterns in adolescence and adulthood.	Shanghai Women's Health Study	33,054	Aged 40-70 years in 1997-2000	Dorjgochoo, Kallianpur et al. Dietary and lifestyle predictors of age at natural menopause and reproductive span in the Shanghai Women's Health Study. *Menopause*. **15**, 924-933.(2008)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Reproductive, birth control	Tubal sterilisation → later menopause	Tubal sterilisation vs no, beta=0.21, p<0.01	Multi-variable linear regression, including age, education, occupation, income, marital status, current employment, energy intake, weight gain between age 20 and 50 years, cigarette smoking (never/ever), and leisure-time physical activity patterns in adolescence and adulthood.	Shanghai Women's Health Study	33,054	Aged 40-70 years in 1997-2000	Dorjgochoo, Kallianpur et al. Dietary and lifestyle predictors of age at natural menopause and reproductive span in the Shanghai Women's Health Study. *Menopause*. **15**, 924-933.(2008)
Reproductive, birth control	Oral contraceptives → later menopause	Oral contraceptives vs no, beta=0.48, p<0.01	Multi-variable linear regression, including age, education, occupation, income, marital status, current employment, energy intake, weight gain between age 20 and 50 years, cigarette smoking (never/ever), and leisure-time physical activity patterns in adolescence and adulthood.	Shanghai Women's Health Study	33,054	Aged 40-70 years in 1997-2000	Dorjgochoo, Kallianpur et al. Dietary and lifestyle predictors of age at natural menopause and reproductive span in the Shanghai Women's Health Study. *Menopause*. **15**, 924-933.(2008)
Reproductive, birth control	Oral contraceptives → later menopause	1+ years oral contraceptive use vs <1 year, HR=0.86 (95% CI 0.77,0.96)	Multivariate Cox PH including parity, age at menarche, oral contraceptive use, unilateral oophorectomy, smoking status, education, vigorous physical activity, and BMI	Black Women's Health Study, USA	17,070	Aged 35-55 in 1995	Palmer, Rosenberg et al. Onset of natural menopause in African American women. *Am J Public Health*. **93**, 299-306.(2003)
Reproductive, birth control	Oral contraceptives → later menopause	Ever used oral contraceptives vs no, HR=0.84 (95% CI 0.76,0.93)	Cox PH, adjusted for smoking, education, marital status, heart disease, parity, race/ethnicity, employment, oral contraceptives	Study of Women's Health Across the Nation (SWAN), USA	14,620	Aged 40-55 in 1995-1997	Gold, Bromberger et al. Factors associated with age at natural menopause in a multiethnic sample of midlife women. *American Journal of Epidemiology*. **153**, 865 - 874.(2001)
Reproductive, birth control	Oral contraceptives → later menopause	Oral contraceptives vs no, HR=0.87 (95% CI 0.81,0.93)	Multivariate Cox PH, adjusted for age, population, education, marital status, smoking, BMI, physical activity, alcohol consumption, supplementation with vitamins and minerals, hormonal contraceptives, HRT.	Health, Alcohol and Psychosocial factors in Eastern Europe (HAPIEE), Russia, Poland, Czech Republic	12,676	Aged 45-69 in 2002-2005	Stepaniak, Szafraniec et al. Age at natural menopause in three central and eastern European urban populations: the HAPIEE study. *Maturitas*. **75**, 87-93.(2013)
Reproductive, birth control	Long-term oral contraceptive use → earlier menopause	Never used as reference, 3+ years HR=1.12 (95% CI 1.03,1.21), 11+ years HR=1.13 (95% CI 1.02,1.25)	Linear regression, Cox PH, adjusted for smoking, parity, BMI, SES, age at menarche.	DOM-3 breast screening programme in Utrecht, The Netherlands	8,701	1932-1941	de Vries, den Tonkelaar et al. Oral contraceptive use in relation to age at menopause in the DOM cohort. *Hum Reprod*. **16**, 1657-1662.(2001)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Reproductive, birth control	Oral contraceptives → later menopause	Oral contraceptives vs none, HR=0.76 (95% CI 0.64,0.91)	Cox PH, adjusted for educational level, age at menarche, menstrual cycle length, oral contraceptives, live births, smoking status, and self-reported health status.	WOMID, Poland	7,183	Aged 35-65 in 2000-2004	Kaczmarek. The timing of natural menopause in Poland and associated factors. *Maturitas*. **57**, 139-153.(2007)
Reproductive, birth control	Oral contraceptives → later menopause	Oral contraceptives vs none, beta=0.09, p=0.00	Multiple linear regression, including age, height, weight, BMI, age at menarche, number of siblings, birth order, age at marriage, fecundity, age at first, childbirth, parity, smoking status, past smoking behavior, current amount of smoking, SES, oral contraceptive use	Doorlopend Onderz oek Morbidi teit/Mortaliteit [DOM], The Netherlands	3,756	1911-1925	van Noord, Dubas et al. Age at natural menopause in a population-based screening cohort: the role of menarche, fecundity, and lifestyle factors. *Fertil Steril*. **68**, 95-102.(1997)
Reproductive, birth control	Tubal sterilization → earlier menopasue	Tubal sterilization vs no, OR=0.38 (95% CI 1.02,1.87)	Logistic regression adjusted for oral contraceptives, smoking, SES. Compared women with younger menopause (<49 years) to those older (≥49 years).	Royal College of General Practitioners' Oral Contraception Study, UK	3,650	Recruited 1968	Pokoradi, Iversen et al. Factors associated with age of onset and type of menopause in a cohort of UK women. *Am J Obstet Gynecol*. **205**, 34.e31-13.(2011)
Reproductive, birth control	Oral contraceptives → earlier menopause	Ever used oral contraceptives vs never, OR=1.37 (95% CI 1.14,1.63)	Logistic regression adjusted for oral contraceptives, smoking, SES. Compared women with younger menopause (<49 years) to those older (≥49 years).	Royal College of General Practitioners' Oral Contraception Study, UK	3,650	Recruited 1968	Pokoradi, Iversen et al. Factors associated with age of onset and type of menopause in a cohort of UK women. *Am J Obstet Gynecol*. **205**, 34.e31-13.(2011)
Reproductive, birth control	Oral contraceptives → later menopause	Oral contraceptives at baseline vs none, HR=0.85 (95% CI 0.75,0.97)	Cox PH adjusted for race/ethnicity, baseline smoking, time-varying smoking, health baseline, educational level, use of oral contraceptives at baseline, alcohol at baseline, alcohol change since baseline, current employment, physical activity, percentile of baseline weight. 10 years follow-up	Study of Women's Health Across the Nation (SWAN), USA	3,302	Aged 40-55 in 1995-1997	Gold, Crawford et al. Factors related to age at natural menopause: longitudinal analyses from SWAN. *Am J Epidemiol*. **178**, 70-83.(2013)
Reproductive, birth control	Oral contraceptives → later menopause	Oral contraceptive vs never used, OR=0.6 (95% CI 0.3,1.0)	Logistic regression (odds of being menopausal) adjusted for age, education, occupation, marital status, history of dysmenorrhea, age at first pregnancy, number of pregnancies, number of deliveries, history of hypertension, oral contraceptive use and HRT use.	Cluster sample of women aged 40-60 years in Ibadan, Nigeria	1,189	Aged 40-60 years in 2006-2007	OlaOlorun and Lawoyin. Age at menopause and factors associated with attainment of menopause in an urban community in Ibadan, Nigeria. *Climacteric*. **12**, 352-363.(2009)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Reproductive, birth control	Oral contraception → earlier menopause	Hormone use menopause ~1.5 years earlier than average, p=0.036	t-test, not adjusted	Women in city of Shiraz, Iran	948	Menopausal in 2000	Ayatollahi, Ghaem et al. Menstrual-reproductive factors and age at natural menopause in Iran. *Int J Gynaecol Obstet*. **80**, 311-313.(2003)
Reproductive, endometriosis	Endometriosis → earlier menopause	Endometriosis vs none, RR=1.33 (95% CI 1.05,1.68)	Multiple logistic regression, adjusted for age, age at menarche, number of pregnancies, BMI, past history of infertility, past history of endometriosis and smoking before menopause. Relative risk (RR) - age adjusted OR.	Japan Nurses' Health Study (JNHS)	24,153	Aged 40-59 years 2001-2007	Yasui, Hayashi et al. Association of endometriosis-related infertility with age at menopause. *Maturitas*. **69**, 279-283.(2011)
Reproductive, endometriosis	Endometriosis → earlier menopause	Endometriosis vs none OR=2.49 (95% CI 1.42,4.37)	Logistic regression adjusted for oral contraceptives, smoking, SES. Compared women with younger menopause (<49 years) to those older (≥49 years).	Royal College of General Practitioners' Oral Contraception Study, UK	3,650	Recruited 1968	Pokoradi, Iversen et al. Factors associated with age of onset and type of menopause in a cohort of UK women. *Am J Obstet Gynecol*. **205**, 34.e31-13.(2011)
Reproductive, fertility	Longer interval between marriage and first child → earlier menopause	Fecundity estimated from (age first childbirth - age at marriage). Beta=-0.04, p=0.05	Multiple linear regression, including age, height, weight, BMI, age at menarche, number of siblings, birth order, age at marriage, fecundity, age at first, childbirth, parity, smoking status, past smoking behavior, current amount of smoking, SES, oral contraceptive use	Doorlopend Onderz oek Morbidi teit/Mortaliteit [DOM], The Netherlands	3,756	1911-1925	van Noord, Dubas et al. Age at natural menopause in a population-based screening cohort: the role of menarche, fecundity, and lifestyle factors. *Fertil Steril*. **68**, 95-102.(1997)
Reproductive, HRT	HRT → later menopause	Ever used HRT vs never, OR=0.28 (95% CI 0.20,0.41)	Logistic regression adjusted for oral contraceptives, smoking, SES. Compared women with younger menopause (<49 years) to those older (≥49 years).	Royal College of General Practitioners' Oral Contraception Study, UK	3,650	Recruited 1968	Pokoradi, Iversen et al. Factors associated with age of onset and type of menopause in a cohort of UK women. *Am J Obstet Gynecol*. **205**, 34.e31-13.(2011)
Reproductive, infertility	Infertility → earlier menopause	Infertility vs no, RR=1.22 (95% CI 1.04,1.44)	Multiple logistic regression, adjusted for age, age at menarche, number of pregnancies, BMI, past history of infertility, past history of endometriosis and smoking before menopause. Relative risk (RR) - age adjusted OR.	Japan Nurses' Health Study (JNHS)	24,153	Aged 40-59 years 2001-2007	Yasui, Hayashi et al. Association of endometriosis-related infertility with age at menopause. *Maturitas*. **69**, 279-283.(2011)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Reproductive, menarche	Earlier menarche → earlier menopause	Aged <11 as reference; HR=0.96 (0.92-0.99) for 11-12 years; HR=0.95 (95% CI 0.91-0.98) for 13-14 years; HR=0.93 (95% CI 0.89-0.97) for 15-16 years; HR=0.92 (95% CI 0.86-0.99) for ≥17 years; p=0.0007 for test of trend.	Age at menopause collected as categories (<45, 45-49, 50-54, ≥55). Mulitvariable Cox PH adjusted for ethnicity, smoking, menarche age, parity, BMI	Multiethnic Cohort Study, USA (non-Latina Whites, Japanese Americans, African Americans, Native Hawai'ians, and Latinas).	95,704	Aged 45-74 years in 1993-1996	Henderson, Bernstein et al. Predictors of the timing of natural menopause in the Multiethnic Cohort Study. *Am J Epidemiol*. **167**, 1287-1294.(2008)
Reproductive, menarche	Earlier menarche → earlier menopause	Menarche ≥16, beta=0.55 vs menarche ≤11. P<0.01.	Multi-variable linear regression, including age, education, occupation, income, marital status, current employment, energy intake, weight gain between age 20 and 50 years, cigarette smoking (never/ever), and leisure-time physical activity patterns in adolescence and adulthood.	Shanghai Women's Health Study	33,054	Aged 40-70 years in 1997-2000	Dorjgochoo, Kallianpur et al. Dietary and lifestyle predictors of age at natural menopause and reproductive span in the Shanghai Women's Health Study. *Menopause*. **15**, 924-933.(2008)
Reproductive, menarche	Earlier menarche → earlier menopause	Menarche ≤11 years, 51.1 years, menarche 12 years, 51.1 years, menarche 13 years, 51.2 years, menarche 14 years, 51.3 years, menarche ≥15 years, 51.3 years; p<0.05	Chi-squared. ANCOVA adjusted for age, education, BMI, smoking, age at menarche, menstrual cycle, parity and oral contraceptive use.	Patients at hospitals in Italy	31,834	Aged ≥55 in 1997-2003	Parazzini. Determinants of age at menopause in women attending menopause clinics in Italy. *Maturitas*. **56**, 280-287.(2007)
Reproductive, menarche	Earlier menarche → earlier menopause	Per year increase in menarche, RR=0.903 (95% CI 0.867,0.940)	Multiple logistic regression, adjusted for age, age at menarche, number of pregnancies, BMI, past history of infertility, past history of endometriosis and smoking before menopause. Relative risk (RR) - age adjusted OR.	Japan Nurses' Health Study (JNHS)	24,153	Aged 40-59 years 2001-2007	Yasui, Hayashi et al. Association of endometriosis-related infertility with age at menopause. *Maturitas*. **69**, 279-283.(2011)
Reproductive, menarche	Earlier menarche → earlier menopause	14+ years vs <14, HR=0.87 (95% CI 0.8,0.94)	Cox PH, multivariate model with education, monthly income, BMI, age at menarche, parity, smoking	Women attending health screening in Jiangsu Province of China	20,275	Aged 40-65 in 2010-2011	Li, Wu et al. Factors associated with the age of natural menopause and menopausal symptoms in Chinese women. *Maturitas*. **73**, 354-360.(2012)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Reproductive, menarche	Earlier menarche → earlier menopause	14+ years vs <14 years, HR=0.82 (95% CI 0.74,0.91)	Cox PH, adjusted for educational level, age at menarche, menstrual cycle length, oral contraceptives, live births, smoking status, and self-reported health status.	WOMID, Poland	7,183	Aged 35-65 in 2000-2004	Kaczmarek. The timing of natural menopause in Poland and associated factors. *Maturitas*. **57**, 139-153.(2007)
Reproductive, menarche	Earlier and later menarche → earlier menopause	Menarche ≤10 years, 50.3 years; 11-14 years, 50.6 years; >14 years, 49.8 years; p=0.005 for log-rank. No significant. diff in Cox PH.	Log-rank. Cox PH adjusted.	Australian Twin Registry	5,961	Aged 17-88 in 1980-82, or aged 50+ in 1993-1995	Do, Treloar et al. Predictive factors of age at menopause in a large Australian twin study. *Hum Biol*. **70**, 1073-1091.(1998)
Reproductive, menarche	Earlier menarche → earlier menopause	(no details in abtract)	Adjusted for SES, smoking, oral contraceptives, HRT	Prospective study, Gothenburg, Sweden	1,017	1930, 1922, 1918, 1914, 1908	Rodstrom, Bengtsson et al. Evidence for a secular trend in menopausal age: a population study of women in Gothenburg. *Menopause*. **10**, 538-543.(2003)
Reproductive, menarche to first birth	Increased time from menarche to first birth → earlier menopause	8+ years beta=-0.19 vs <8 years, p<0.01	Multi-variable linear regression, including age, education, occupation, income, marital status, current employment, energy intake, weight gain between age 20 and 50 years, cigarette smoking (never/ever), and leisure-time physical activity patterns in adolescence and adulthood.	Shanghai Women's Health Study	33,054	Aged 40-70 years in 1997-2000	Dorjgochoo, Kallianpur et al. Dietary and lifestyle predictors of age at natural menopause and reproductive span in the Shanghai Women's Health Study. *Menopause*. **15**, 924-933.(2008)
Reproductive, menstrual cycle	Menstrual cycle irregularity → later menopause	Regular 51.2 years vs irregular 51.4 years, p<0.05	Chi-squared. ANCOVA adjusted for age, education, BMI, smoking, age at menarche, menstrual cycle, parity and oral contraceptive use.	Patients at hospitals in Italy	31,834	Aged ≥55 in 1997-2003	Parazzini. Determinants of age at menopause in women attending menopause clinics in Italy. *Maturitas*. **56**, 280-287.(2007)
Reproductive, menstrual cycle	Menstrual cycle irregularity → later menopause	Cycle irregularity HR=0.778 (95% CI 0.722,0.839)	Multivariate Cox PH, endpoints menopause, early menopause (<45 years), premature ovarian failure (<40 years). Adjusted for age at menarche, number of deliveries, current BMI, cycle regularity, unilateral oophorectomy, oral contraceptives, ever smoker before menopause and birth year decade.	Japan Nurses' Health Study (JNHS)	24,152	Aged 40-59 years 2001-2007	Yasui, Hayashi et al. Factors associated with premature ovarian failure, early menopause and earlier onset of menopause in Japanese women. *Maturitas*. **72**, 249-255.(2012)
Reproductive, menstrual cycle	Shorter menstrual cycle → earlier menopause	Under 28 days as reference, 28-32 days HR =0.90 (95% CI 0.81,0.99), >32 days HR=0.86 (95% CI 0.76,0.96)	Cox PH, adjusted for educational level, age at menarche, menstrual cycle length, oral contraceptives, live births, smoking status, and self-reported health status.	WOMID, Poland	7,183	Aged 35-65 in 2000-2004	Kaczmarek. The timing of natural menopause in Poland and associated factors. *Maturitas*. **57**, 139-153.(2007)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Reproductive, menstrual cycle	Longer time from menarche to regular menses → later menopause	5+ years from menarche to regular menses, HR=0.67 (95% CI 0.50-0.89) vs <1 year	Cox PH adjusted for age.	European prospective investigation into cancer and nutrition (EPIC) - Heidelberg, Germany	4,807	Aged 35-65 in 1994-1998	Nagel, Altenburg et al. Reproductive and dietary determinants of the age at menopause in EPIC-Heidelberg. *Maturitas*. **52**, 337-347.(2005)
Reproductive, menstrual cycle	Menstrual cycle irregularity before age 25 → later menopause	Iregularity <25 yrs vs regular, beta(hazard)=-0.4 years, p=0.0001	Multivariate stepwise PH regression including geographic area, marital status, income, education, history of irregular menstrual periods, parity, and ever use of oral contraceptives. Comparison of medians, Mantel-Cox test for differences.	Breast Cancer Detection Demonstration Project (BCDDP), USA	1,423	Aged 22-62 years in 1973-1977	Stanford, Hartge et al. Factors influencing the age at natural menopause. *J Chronic Dis*. **40**, 995-1002.(1987)
Reproductive, menstrual cycle	Shorter menstrual cycle → earlier menopause	Menstrual cycle <26 days vs 26-32 days, OR=1.62 (95% CI 1.06,2.47)	Discrete-time Cox survival - logistic regression, odds of natural menopause for each year of age over the interval 44-56 years, adjusted for parity, age of menarche	Menstruation and Reproductive History Study, USA	561	Under 25 years in 1935-1939	Whelan, Sandler et al. Menstrual and reproductive characteristics and age at natural menopause. *Am J Epidemiol*. **131**, 625-632.(1990)
Reproductive, number of births	More births → later menopause	No births as reference; 1 birth HR=0.94 (95% CI 0.91,0.97); 2-3 births HR=0.88 (95% CI 0.85,0.90); ≥4 births, HR=0.88 (95% CI 0.85,0.90) ; p<0.0001	Age at menopause collected as categories (<45, 45-49, 50-54, ≥55). Mulitvariable Cox PH adjusted for ethnicity, smoking, menarche age, parity, BMI	Multiethnic Cohort Study, USA (non-Latina Whites, Japanese Americans, African Americans, Native Hawai'ians, and Latinas).	95,704	Aged 45-74 years in 1993-1996	Henderson, Bernstein et al. Predictors of the timing of natural menopause in the Multiethnic Cohort Study. *Am J Epidemiol*. **167**, 1287-1294.(2008)
Reproductive, number of births	More births → later menopause	For 3+ births vs 0 births, HR=0.84 (95% CI 0.81,0.87); p<0.001 for trend	Competing risks Cox PH adjusted for age, smoking status, BMI at 40 years.	Breakthrough Generations Study, UK	50,678	Aged 40-98 in 2003-2011	Morris, Jones et al. Body mass index, exercise, and other lifestyle factors in relation to age at natural menopause: analyses from the breakthrough generations study. *Am J Epidemiol*. **175**, 998-1005.(2012)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Reproductive, number of births	More births → later menopause	For 4+ births vs 0 births, beta=0.73, p<0.01	Multi-variable linear regression, including age, education, occupation, income, marital status, current employment, energy intake, weight gain between age 20 and 50 years, cigarette smoking (never/ever), and leisure-time physical activity patterns in adolescence and adulthood.	Shanghai Women's Health Study	33,054	Aged 40-70 years in 1997-2000	Dorjgochoo, Kallianpur et al. Dietary and lifestyle predictors of age at natural menopause and reproductive span in the Shanghai Women's Health Study. *Menopause*. **15**, 924-933.(2008)
Reproductive, number of births	More births → later menopause	No births 51.0 years, 1 birth 51.1 years, 2+ births 51.2 years, p<0.05	Chi-squared. ANCOVA adjusted for age, education, BMI, smoking, age at menarche, menstrual cycle, parity and oral contraceptive use.	Patients at hospitals in Italy	31,834	Aged ≥55 in 1997-2003	Parazzini. Determinants of age at menopause in women attending menopause clinics in Italy. *Maturitas*. **56**, 280-287.(2007)
Reproductive, number of births	More births → later menopause	≥1 delivery vs 0, HR(early menopause)=0.683 (95% CI 0.486,0.960)	Multivariate Cox PH, endpoints menopause, early menopause (<45 years), premature ovarian failure (<40 years). Adjusted for age at menarche, number of deliveries, current BMI, cycle regularity, unilateral oophorectomy, oral contraceptives, ever smoker before menopause and birth year decade.	Japan Nurses' Health Study (JNHS)	24,152	Aged 40-59 years 2001-2007	Yasui, Hayashi et al. Factors associated with premature ovarian failure, early menopause and earlier onset of menopause in Japanese women. *Maturitas*. **72**, 249-255.(2012)
Reproductive, number of births	More births → later menopause	2+ births 48.6 years (p<0.0001) vs 0 births	Multiple linear regression of geographical region, race,age, parity, smoking, region, income, education, physical activity, and history of CVD	REasons for Geographic And Racial Differences in Stroke and Myocardial Infarction (REGARDS), USA	22,484	Aged >=45 in 2003-2007)	McKnight, Wellons et al. Racial and regional differences in age at menopause in the United States: findings from the REasons for Geographic And Racial Differences in Stroke (REGARDS) study. *Am J Obstet Gynecol*. **205**, 353.e351-358.(2011)
Reproductive, number of births	More births → later menopause	2+ births vs 0 births, HR=0.53 (95% CI 0.32,0.87). p<0.001 for trend.	Cox PH, multivariate model with education, monthly income, BMI, age at menarche, parity, smoking	Women attending health screening in Jiangsu Province of China	20,275	Aged 40-65 in 2010-2011	Li, Wu et al. Factors associated with the age of natural menopause and menopausal symptoms in Chinese women. *Maturitas*. **73**, 354-360.(2012)
Reproductive, number of births	No births → earlier menopause	Any live births, HR=0.80 (95% CI 0.70-0.93)	Cox PH, adjusted for smoking, education, marital status, heart disease, parity, race/ethnicity, employment, oral contraceptives	Study of Women's Health Across the Nation (SWAN), USA	14,620	Aged 40-55 in 1995-1997	Gold, Bromberger et al. Factors associated with age at natural menopause in a multiethnic sample of midlife women. *American Journal of Epidemiology*. **153**, 865 - 874.(2001)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Reproductive, number of births	More births → later menopause	Association of number of children (0, 1-2, 3+) p<0.001	Log-rank test, unadjusted	DOM-3 breast screening programme in Utrecht, The Netherlands	8,701	1932-1941	de Vries, den Tonkelaar et al. Oral contraceptive use in relation to age at menopause in the DOM cohort. *Hum Reprod*. **16**, 1657-1662.(2001)
Reproductive, number of births	More births → later menopause	3+ live births HR=0.71 (95% CI 0.52-0.98); p=0.04 for trend	Cox PH, adjusted for educational level, age at menarche, menstrual cycle length, oral contraceptives, live births, smoking status, and self-reported health status.	WOMID, Poland	7,183	Aged 35-65 in 2000-2004	Kaczmarek. The timing of natural menopause in Poland and associated factors. *Maturitas*. **57**, 139-153.(2007)
Reproductive, number of births	More births → later menopause	1-2 births, HR=0.83 (95% CI 0.71,0.99), >2 births HR=0.77 (95% CI 0.66,0.89) vs 0 births. P=0.000 for log-rank test.	Log-rank. Cox PH adjusted.	Australian Twin Registry	5,961	Aged 17-88 in 1980-82, or aged 50+ in 1993-1995	Do, Treloar et al. Predictive factors of age at menopause in a large Australian twin study. *Hum Biol*. **70**, 1073-1091.(1998)
Reproductive, number of births	No births/one birth → later menopause	0/1 births vs 2+, HR=0.744 (95% CI 0.619,0.894).	Cox PH, adjusted for country	European Respiratory Health Survey (Spain, France, Belgium, Switzerland, UK, Norway, Sweden, Iceland, and Estonia) and the Swiss Air Pollution and Lung Disease in Adults Cohort	5,288	Aged 30-60 1998-2002	Dratva, Gomez Real et al. Is age at menopause increasing across Europe? Results on age at menopause and determinants from two population-based studies. *Menopause*. **16**, 385-394.(2009)
Reproductive, number of births	More births → later menopause	1 or 2 births vs 0 births, HR=0.74 (95% CI 0.62,0.87)	Cox PH adjusted for age.	European prospective investigation into cancer and nutrition (EPIC) - Heidelberg, Germany	4,807	Aged 35-65 in 1994-1998	Nagel, Altenburg et al. Reproductive and dietary determinants of the age at menopause in EPIC-Heidelberg. *Maturitas*. **52**, 337-347.(2005)
Reproductive, number of births	More births → later menopause	3+ births vs 0, HR=0.78 (95% CI 0.67,0.90)	Cox PH, mutivariate model of age, parity, religion, BMI, cigarettes per day.	New York University Women Study, USA	4,694	Mean age 42.8 years in 1985-1991	Kato, Toniolo et al. Prospective study of factors influencing the onset of natural menopause.[see comment]. *Journal of Clinical Epidemiology*. **51**, 1271 - 1276.(1998)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Reproductive, number of births	More births → later menopause	0 births, 50.0 years; 1 birth, 50.4 years; 2 births, 50.6 years; 3 births, 50.9 years; 4 births, 51.2 years; 5 births, 50.9 years (p<0.01)	ANCOVA - adjusted for cohort of birth, BMI, smoking, age at menarche, number of births	Italian Climacteric Research Group Study (ICARUS) - prospective study of effect of menopause on womens' health	4,300	Aged >=55 in 1997	Meschia, Pansini et al. Determinants of age at menopause in Italy: results from a large cross-sectional study. *Maturitas*. **34**, 119-125.(2000)
Reproductive, number of births	No births → earlier menopause	No births HR=1.14 (95% CI 1.02,1.28)	Cox PH	Women from 7 cities in Latin America and the Caribbean	4,056	Aged 60-79 years	Velez, Alvarado et al. Life course socioeconomic adversity and age at natural menopause in women from Latin America and the Caribbean. *Menopause*. **17**, 552-559.(2010)
Reproductive, number of births	More births → earlier menopause	5+ births HR=1.15 (95% CI= 1.06,1.24)	Cox PH	Women from 7 cities in Latin America and the Caribbean	4,056	Aged 60-79 years	Velez, Alvarado et al. Life course socioeconomic adversity and age at natural menopause in women from Latin America and the Caribbean. *Menopause*. **17**, 552-559.(2010)
Reproductive, number of births	More births → later menopause	2.40 births for early vs 2.58 for late, p=0.035	Chi-squared, or t-test. Not adjusted. Early menopause (<49 years) vs. late menopause (≥53 years)	Doorlopend Onderz oek Morbidi teit/Mortaliteit [DOM], The Netherlands	3,756	1911-1925	van Noord, Dubas et al. Age at natural menopause in a population-based screening cohort: the role of menarche, fecundity, and lifestyle factors. *Fertil Steril*. **68**, 95-102.(1997)
Reproductive, number of births	More births → later menopause	For menopause before/after 50, p=0.001	Cox PH, menopause before/after 50 years considered separately	Medical Research Council National Survey of Health and Development (1946 Birth Cohort)	1,583	1946	Mishra, Hardy et al. Are the effects of risk factors for timing of menopause modified by age? Results from a British birth cohort study. *Menopause*. **14**, 717-724.(2007)
Reproductive, number of births	More births → later menopause	Beta (hazard) -0.07 years. P=0.0001	Multivariate stepwise PH regression including geographic area, marital status, income, education, history of irregular menstrual periods, parity, and ever use of oral contraceptives. Comparison of medians, Mantel-Cox test for differences.	Breast Cancer Detection Demonstration Project (BCDDP), USA	1,423	Aged 22-62 years in 1973-1977	Stanford, Hartge et al. Factors influencing the age at natural menopause. *J Chronic Dis*. **40**, 995-1002.(1987)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Reproductive, number of births	No births → earlier menopause	Nulliparous menopause ~1.5 years earlier than average, p=0.007	ANOVA, not adjusted	Women in city of Shiraz, Iran	948	Menopausal in 2000	Ayatollahi, Ghaem et al. Menstrual-reproductive factors and age at natural menopause in Iran. *Int J Gynaecol Obstet*. **80**, 311-313.(2003)
Reproductive, number of births	More births → later menopause	5+ births vs 0, OR=0.54 (95% CI 0.34,0.85)	Discrete-time Cox survival - logistic regression, odds of natural menopause for each year of age over the interval 44-56 years, adjusted for age of menarche, median cycle length	Menstruation and Reproductive History Study, USA	561	Under 25 years in 1935-1939	Whelan, Sandler et al. Menstrual and reproductive characteristics and age at natural menopause. *Am J Epidemiol*. **131**, 625-632.(1990)
Reproductive, number of births	More births → later menopause	4+ births vs 0, HR=0.33 (95% CI 0.14,0.79)	Cox PH adjusted for education, parity, marital status, BMI, smoking, alcohol consumption, age at menarche and oral contraceptive use for Spanish and Latin-American models. For Latin-American models, also place of birth and whether or not women had experienced menopause in their country of origin	Decisions at Menopause Study (2002–2003), Madrid, Spain	484	Aged 45-55 in 2002-2003; Aged 45-55 in 2010-2011	Perez-Alcala, Sievert et al. Cross cultural analysis of factors associated with age at natural menopause among Latin-American immigrants to Madrid and their Spanish neighbors. *Am J Hum Biol*. **25**, 780-788.(2013)
Reproductive, number of births	More births → later menopause	Increasing births, HR=0.92 (95% CI 0.84,0.996)	Cox PH, adjusted	Women living in Tuzla, Bosnia and Herzegovina during war in 1992-1995 and up until interview	331	Aged 39-75 years in 2009-2011	Balic, Rizvanovic et al. Age at natural menopause in refugee and domicile women who lived in Tuzla Canton in Bosnia and Herzegovina during and after the war. *Menopause*. **21**, 721-725.(2014)
Reproductive, number of pregnancies	More pregnancies → later menopause	Per pregnancy, RR=0.963 (95% CI 0.928,1.000)	Multiple logistic regression, adjusted for age, age at menarche, number of pregnancies, BMI, past history of infertility, past history of endometriosis and smoking before menopause. Relative risk (RR) - age adjusted OR.	Japan Nurses' Health Study (JNHS)	24,153	Aged 40-59 years 2001-2007	Yasui, Hayashi et al. Association of endometriosis-related infertility with age at menopause. *Maturitas*. **69**, 279-283.(2011)
Reproductive, number of pregnancies	Never pregnant → earlier menopause	Higher proportion of 0 pregnancies for menopause <40 years, p=0.003	Chi-squared test against age at menopause in 4 categories (<40, 40-44, 45-49, ≥50)	National Health and Nutrition Examination Survey (NHANES), USA	3,191	Aged 25-74 years in 1971-1975	Cooper and Sandler. Age at natural menopause and mortality. *Ann Epidemiol*. **8**, 229-235.(1998)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Reproductive, number of pregnancies	Never pregnant → earlier menopause	2 pregnancies vs null, HR=0.68 (95% CI 0.526,0.879)	Cox PH analysis in women with menopause <52 years and 52+ years, adjusted for smoking status, parity, age at menarche, oral contraceptive, education, BMI, physical activity, language region	SAPALDIA Swiss cohort study on Air Pollution and Lung Diseases in Adults (SAPALDIA)	3,119	Aged 18-60 years in 1992	Dratva, Zemp et al. Variability of reproductive history across the Swiss SAPALDIA cohort--patterns and main determinants. *Ann Hum Biol*. **34**, 437-453.(2007)
Reproductive, number of pregnancies	Never pregnant → earlier menopause	0 pregnancies ~1.5 years earlier than average, p=0.002	ANOVA, not adjusted	Women in city of Shiraz, Iran	948	Menopausal in 2000	Ayatollahi, Ghaem et al. Menstrual-reproductive factors and age at natural menopause in Iran. *Int J Gynaecol Obstet*. **80**, 311-313.(2003)
Reproductive, number of pregnancies	Never pregnant → earlier menopause	1+ pregnancies, OR=0.72 (95% CI 0.53,0.98)	Discrete-time Cox survival - logistic regression, odds of natural menopause for each year of age over the interval 44-56 years, adjusted for age of menarche, median cycle length	Menstruation and Reproductive History Study, USA	561	Under 25 years in 1935-1939	Whelan, Sandler et al. Menstrual and reproductive characteristics and age at natural menopause. *Am J Epidemiol*. **131**, 625-632.(1990)
Reproductive, number of pregnancies	More pregnancies → later menopause	5+ pregnancies, OR=0.65 (95% CI 0.45,0.95)	Discrete-time Cox survival - logistic regression, odds of natural menopause for each year of age over the interval 44-56 years, adjusted for age of menarche, median cycle length	Menstruation and Reproductive History Study, USA	561	Under 25 years in 1935-1939	Whelan, Sandler et al. Menstrual and reproductive characteristics and age at natural menopause. *Am J Epidemiol*. **131**, 625-632.(1990)
Reproductive, time spent breastfeeding	Breastfed children → later menopause	Breastfeeding for 1-5 months vs none HR=0.81 (95% CI 0.69,0.94)	Cox PH adjusted for age.	European prospective investigation into cancer and nutrition (EPIC) - Heidelberg, Germany	4,807	Aged 35-65 in 1994-1998	Nagel, Altenburg et al. Reproductive and dietary determinants of the age at menopause in EPIC-Heidelberg. *Maturitas*. **52**, 337-347.(2005)
Reproductive, unilateral oophorectomy	Unilateral oophorectomy → earlier menopause	Unilateral oophorectomy: for quantitative trait, HR=1.40 (95% CI 1.22,1.60). POF, HR=3.32 (95% CI 1.42,7.77). EM, HR=3.94 (95% CI 2.63,5.89)	Multivariate Cox PH, endpoints menopause, early menopause (<45 years), premature ovarian failure (<40 years). Adjusted for age at menarche, number of deliveries, current BMI, cycle regularity, unilateral oophorectomy, oral contraceptives, ever smoker before menopause and birth year decade.	Japan Nurses' Health Study (JNHS)	24,152	Aged 40-59 years 2001-2007	Yasui, Hayashi et al. Factors associated with premature ovarian failure, early menopause and earlier onset of menopause in Japanese women. *Maturitas*. **72**, 249-255.(2012)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Reproductive, unilateral oophorectomy	Unilateral oophorectomy → earlier menopause	Adjusted relative risk 1.27 (95% CI: 1.14,1.41)	Cox PH adjusted for birth cohort, parity, smoking, BMI, age at menarche	HUNT2 (Nord-Trøndelag Health Study), Norway	23,580	1925-1977	Bjelland, Wilkosz et al. Is unilateral oophorectomy associated with age at menopause? A population study (the HUNT2 Survey). *Hum Reprod*. **29**, 835-841.(2014)
Season of birth	Spring → earlier menopause; Autumn → later menopause	Spring vs autumn, beta = -0.85, p=0.0002	Multiple regression - age at menarche, BMI, smoking, education, employment	Women attending three Italian menopause clinics	2,822	Mean age of 53 years in 1997-2001	Cagnacci, Pansini et al. Season of birth influences the timing of menopause. *Hum Reprod*. **20**, 2190-2193.(2005)
SES	Lower SES → earlier menopause	Health insurance type, p<0.001 for association.	Log-rank test, unadjusted	DOM-3 breast screening programme in Utrecht, The Netherlands	8,701	1932-1941	de Vries, den Tonkelaar et al. Oral contraceptive use in relation to age at menopause in the DOM cohort. *Hum Reprod*. **16**, 1657-1662.(2001)
SES	Lower SES → earlier menopause	p=0.0007 for association with class in log-rank. Not significant. in Cox PH.	Log-rank. Cox PH adjusted.	Australian Twin Registry	5,961	Aged 17-88 in 1980-82, or aged 50+ in 1993-1995	Do, Treloar et al. Predictive factors of age at menopause in a large Australian twin study. *Hum Biol*. **70**, 1073-1091.(1998)
SES	Lower SES → earlier menopause	Increasing SES, beta=0.04, p=0.03	Multiple linear regression, including age, height, weight, BMI, age at menarche, number of siblings, birth order, age at marriage, fecundity, age at first, childbirth, parity, smoking status, past smoking behavior, current amount of smoking, SES, oral contraceptive use	Doorlopend Onderz oek Morbidi teit/Mortaliteit [DOM], The Netherlands	3,756	1911-1925	van Noord, Dubas et al. Age at natural menopause in a population-based screening cohort: the role of menarche, fecundity, and lifestyle factors. *Fertil Steril*. **68**, 95-102.(1997)
SES	Lower SES → earlier menopause	SES 1.5 for early vs 1.7 for late	Chi-squared, or t-test. Not adjusted. Early menopause (<49 years) vs. late menopause (≥53 years).	Doorlopend Onderz oek Morbidi teit/Mortaliteit [DOM], The Netherlands	3,756	1911-1925	van Noord, Dubas et al. Age at natural menopause in a population-based screening cohort: the role of menarche, fecundity, and lifestyle factors. *Fertil Steril*. **68**, 95-102.(1997)
SES	Lower SES → earlier menopause	Manual vs non-manual class as adult, -0.55 years (95% CI -0.94, 0.17)	Multiple linear regression, adjusting for age, age menarche, nulliparity, every use of oral contraception, use of hormone replacement therapy, smoking, BMI (quadratic term included) and all indicators of childhood socio-economic position	British Women's Heart and Health Study	3,513	Aged 60-79 years in 1999-2001	Lawlor, Ebrahim et al. The association of socio-economic position across the life course and age at menopause: the British Women's Heart and Health Study. *Bjog*. **110**, 1078-1087.(2003)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
SES, employment	Not employed → earlier menopause	Not employed vs employed HR=1.20 (95% CI 1.08,1.33)	Cox PH, adjusted for smoking, education, marital status, heart disease, parity, race/ethnicity, employment, oral contraceptives	Study of Women's Health Across the Nation (SWAN), USA	14,620	Aged 40-55 in 1995-1997	Gold, Bromberger et al. Factors associated with age at natural menopause in a multiethnic sample of midlife women. *American Journal of Epidemiology*. **153**, 865 - 874.(2001)
SES, employment	Not employed → earlier menopause	Being employed during follow-up, HR = 0.87 (95% CI 0.77,0.98)	Cox PH adjusted for race/ethnicity, baseline smoking, time-varying smoking, health baseline, educational level, use of oral contraceptives at baseline, alcohol at baseline, alcohol change since baseline, current employment, physical activity, percentile of baseline weight. 10 years follow-up	Study of Women's Health Across the Nation (SWAN), USA	3,302	Aged 40-55 in 1995-1997	Gold, Crawford et al. Factors related to age at natural menopause: longitudinal analyses from SWAN. *Am J Epidemiol*. **178**, 70-83.(2013)
SES, housing tenure	Non-home owner → earlier menopause	Non home owner at 2 of 3 timepoints (26, 36, 43 years), HR(unadjusted)=1.52 (95% CI 1.14,2.04). HR=1.15 (95% CI 1.02,1.30) in model of childhood social class, adult housing tenure, and hardship as adult.	Cox PH, adjusted (i) for adult lifestyle (smoking, BMI, parity, marital status) (ii) psychological health (iii) childhood factors (cognitive score at age 8 years, breast feeding).	Medical Research Council National Survey of Health and Development (1946 Birth Cohort)	1,515	1946	Hardy and Kuh. Social and environmental conditions across the life course and age at menopause in a British birth cohort study. *Bjog*. **112**, 346-354.(2005)
SES, income	Lower income → earlier menopause	Highest income category vs lowest 48.2 years (p=0.03)	Multiple linear regression of geographical region, race, age, parity, smoking, region, income, education, physical activity, and history of CVD	REasons for Geographic And Racial Differences in Stroke and Myocardial Infarction (REGARDS), USA	22,484	Aged >=45 in 2003-2007)	McKnight, Wellons et al. Racial and regional differences in age at menopause in the United States: findings from the REasons for Geographic And Racial Differences in Stroke (REGARDS) study. *Am J Obstet Gynecol*. **205**, 353.e351-358.(2011)
SES, income	Lower income → earlier menopause	Highest monthly income vs lowest, HR=0.79 (95% CI 0.69,0.91). 4 categories, p=0.003 for trend	Cox PH, multivariate model with education, monthly income, BMI, age at menarche, parity, smoking	Women attending health screening in Jiangsu Province of China	20,275	Aged 40-65 in 2010-2011	Li, Wu et al. Factors associated with the age of natural menopause and menopausal symptoms in Chinese women. *Maturitas*. **73**, 354-360.(2012)
SES, income	Higher income → later menopause	p=0.0001 for association with income in log-rank.	Log-rank.	Australian Twin Registry	5,961	Aged 17-88 in 1980-82, or aged 50+ in 1993-1995	Do, Treloar et al. Predictive factors of age at menopause in a large Australian twin study. *Hum Biol*. **70**, 1073-1091.(1998)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
SES, income	Financial hardship → later menopause	Hardship at 36 and 43 years vs none, HR(unadjusted)=0.58 (95% CI 0.36,0.95). HR=0.73 (95% CI 0.59,0.91) in model of childhood social class, adult housing tenure, and hardship as adult.	Cox PH, adjusted (i) for adult lifestyle (smoking, BMI, parity, marital status) (ii) psychological health (iii) childhood factors (cognitive score at age 8 years, breast feeding).	Medical Research Council National Survey of Health and Development (1946 Birth Cohort)	1,515	1946	Hardy and Kuh. Social and environmental conditions across the life course and age at menopause in a British birth cohort study. *Bjog*. **112**, 346-354.(2005)
SES, income	Lower income → earlier menopause	Increasing income, beta (hazard)=-0.08 years, p=0.003	Multivariate stepwise PH regression including geographic area, marital status, income, education, history of irregular menstrual periods, parity, and ever use of oral contraceptives. Comparison of medians, Mantel-Cox test for differences.	Breast Cancer Detection Demonstration Project (BCDDP), USA	1,423	Aged 22-62 years in 1973-1977	Stanford, Hartge et al. Factors influencing the age at natural menopause. *J Chronic Dis*. **40**, 995-1002.(1987)
SES, lifetime	More lifetime adversity → earlier menopause	6 indicators of lifetime adversity vs 0 indicators, HR=1.4 (95% CI 1.10,1.77) .	Cox PH	Women from 7 cities in Latin America and the Caribbean	4,056	Aged 60-79 years	Velez, Alvarado et al. Life course socioeconomic adversity and age at natural menopause in women from Latin America and the Caribbean. *Menopause*. **17**, 552-559.(2010)
SES, lifetime	More adverse indicators across life course → earlier menopause	Number of adverse SES indicators, 9-10 vs 0-1 as reference, effect -1.72 years (95% CI -3.06,-0.39), p<0.001 for trend.	Multiple linear regression, adjusting for age, age menarche, nulliparity, every use of oral contraception, use of hormone replacement therapy, smoking, BMI (quadratic term included)	British Women's Heart and Health Study	3,513	Aged 60-79 years in 1999-2001	Lawlor, Ebrahim et al. The association of socio-economic position across the life course and age at menopause: the British Women's Heart and Health Study. *Bjog*. **110**, 1078-1087.(2003)
SES, occupation	Lower occupational class → earlier menopause	Blue collar vs upper white collar, HR=1.23 (95% CI 1.05,1.45). p=0.0004 log-rank test.	Log-rank. Cox PH adjusted.	Australian Twin Registry	5,961	Aged 17-88 in 1980-82, or aged 50+ in 1993-1995	Do, Treloar et al. Predictive factors of age at menopause in a large Australian twin study. *Hum Biol*. **70**, 1073-1091.(1998)
SES, occupation	Lower occupational class → earlier menopause	Manual occupation/housewife HR=1.12 (95% CI 1.03,1.20)	Cox PH	Women from 7 cities in Latin America and the Caribbean	4,056	Aged 60-79 years	Velez, Alvarado et al. Life course socioeconomic adversity and age at natural menopause in women from Latin America and the Caribbean. *Menopause*. **17**, 552-559.(2010)
SES, occupation	Lower occupational class → earlier menopause	Lower white-collar as reference. Upper white-collar OR=0.74 (95% CI 0.57,0.96); blue collar service workers, OR=1.22 (95% CI 1.01,1.47); blue collar factory workers, OR=1.21 (95% CI 1.01,1.46)	Cox PH multivariate model including smoking, hormone use, BMI, age at first full-term pregnancy	National Finnish Register	1,505	Aged 45-64 in 1989	Luoto, Kaprio et al. Age at natural menopause and sociodemographic status in Finland. *Am J Epidemiol*. **139**, 64-76.(1994)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
SES, occupation	Higher occupational class → later menopause	33% managerial/professional worker menopausal vs 49% routine/manual, p<0.0001	Logistic regression (odds of being menopausal) adjusted for age, education, occupation, marital status, history of dysmenorrhea, age at first pregnancy, number of pregnancies, number of deliveries, history of hypertension, oral contraceptive use and HRT use.	Cluster sample of women aged 40-60 years in Ibadan, Nigeria	1,189	Aged 40-60 years in 2006-2007	OlaOlorun and Lawoyin. Age at menopause and factors associated with attainment of menopause in an urban community in Ibadan, Nigeria. *Climacteric*. **12**, 352-363.(2009)
Smoking	Ever smoker → earlier menopause	Ever smoked vs never, RR=1.34 (95% CI 1.19,1.51)	Multiple logistic regression, adjusted for age, age at menarche, number of pregnancies, BMI, past history of infertility, past history of endometriosis and smoking before menopause. Relative risk (RR) - age adjusted OR.	Japan Nurses' Health Study (JNHS)	24,153	Aged 40-59 years 2001-2007	Yasui, Hayashi et al. Association of endometriosis-related infertility with age at menopause. *Maturitas*. **69**, 279-283.(2011)
Smoking	Smoking → earlier menopause	0 pack-years as reference, 15-30 pack-years OR=1.75 (95% CI 1.36,2.25), 30+ pack-years OR=1.71 (95% CI 1.30,2.24)	Logistic regression adjusted for oral contraceptives, smoking, SES. Compared women with younger menopause (<49 years) to those older (≥49 years).	Royal College of General Practitioners' Oral Contraception Study, UK	3,650	Recruited 1968	Pokoradi, Iversen et al. Factors associated with age of onset and type of menopause in a cohort of UK women. *Am J Obstet Gynecol*. **205**, 34.e31-13.(2011)
Smoking	Smoking → earlier menopause	Smoking (time-varying variable) vs never, HR=4.53 (95% CI 1.18,2.00)	Cox PH adjusted for race/ethnicity, baseline smoking, time-varying smoking, health baseline, educational level, use of oral contraceptives at baseline, alcohol at baseline, alcohol change since baseline, current employment, physical activity, percentile of baseline weight. 10 years follow-up	Study of Women's Health Across the Nation (SWAN), USA	3,302	Aged 40-55 in 1995-1997	Gold, Crawford et al. Factors related to age at natural menopause: longitudinal analyses from SWAN. *Am J Epidemiol*. **178**, 70-83.(2013)
Smoking, current	Current smoking → earlier menopause	Current smoker vs never, HR=1.34 (95% CI 1.28,1.41)	Competing risks Cox PH adjusted for age, parity, BMI at 40 years.	Breakthrough Generations Study, UK	50,678	Aged 40-98 in 2003-2011	Morris, Jones et al. Body mass index, exercise, and other lifestyle factors in relation to age at natural menopause: analyses from the breakthrough generations study. *Am J Epidemiol*. **175**, 998-1005.(2012)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Smoking, current	Longer smoking → earlier menopause	<28 years beta=-0.51, 28+ years beta=-0.86, vs never smoked, p<0.01	Multi-variable linear regression, including age, education, occupation, income, marital status, current employment, energy intake, weight gain between age 20 and 50 years, cigarette smoking (never/ever), and leisure-time physical activity patterns in adolescence and adulthood.	Shanghai Women's Health Study	33,054	Aged 40-70 years in 1997-2000	Dorjgochoo, Kallianpur et al. Dietary and lifestyle predictors of age at natural menopause and reproductive span in the Shanghai Women's Health Study. *Menopause*. **15**, 924-933.(2008)
Smoking, current	Current smoking → earlier menopause	Current smoker beta=-0.76, former beta =-0.4, vs never, p<0.01	Multi-variable linear regression, including age, education, occupation, income, marital status, current employment, energy intake, weight gain between age 20 and 50 years, cigarette smoking (never/ever), and leisure-time physical activity patterns in adolescence and adulthood.	Shanghai Women's Health Study	33,054	Aged 40-70 years in 1997-2000	Dorjgochoo, Kallianpur et al. Dietary and lifestyle predictors of age at natural menopause and reproductive span in the Shanghai Women's Health Study. *Menopause*. **15**, 924-933.(2008)
Smoking, current	Current smoking → earlier menopause	Smokers 51.1 years vs non-smokers 51.2 years, p<0.05	Chi-squared. ANCOVA adjusted for age, education, BMI, smoking, age at menarche, menstrual cycle, parity and oral contraceptive use.	Patients at hospitals in Italy	31,834	Aged ≥55 in 1997-2003	Parazzini. Determinants of age at menopause in women attending menopause clinics in Italy. *Maturitas*. **56**, 280-287.(2007)
Smoking, current	Current smoking → earlier menopause	Current smoker, 47.1 years vs 48.7 years for never (p<0.0001)	Multiple linear regression of geographical region, race,age, parity, smoking, region, income, education, physical activity, and history of CVD	REasons for Geographic And Racial Differences in Stroke and Myocardial Infarction (REGARDS), USA	22,484	Aged >=45 in 2003-2007)	McKnight, Wellons et al. Racial and regional differences in age at menopause in the United States: findings from the REasons for Geographic And Racial Differences in Stroke (REGARDS) study. *Am J Obstet Gynecol*. **205**, 353.e351-358.(2011)
Smoking, current	Smoking around time of menopause → earlier menopause	Smoking at age 40-49 years vs nonsmoker, HR=1.40 (95% CI 1.31,1.49)	Multivariate Cox PH including age, race/ethnicity, education, childhood family income, smoking status in 40s	Sister Study, USA (US and Puerto Rican women)	22,165	Aged 35-74 years in 2003-2007	Steiner, D'Aloisio et al. Association of intrauterine and early-life exposures with age at menopause in the sister study. *American Journal of Epidemiology*. **172**, 140-148.(2010)
Smoking, current	Current smoking → earlier menopause	Current smoking HR=1.47 (95% CI 1.12, 1.92)	Cox PH, multivariate model with education, monthly income, BMI, age at menarche, parity, smoking	Women attending health screening in Jiangsu Province of China	20,275	Aged 40-65 in 2010-2011	Li, Wu et al. Factors associated with the age of natural menopause and menopausal symptoms in Chinese women. *Maturitas*. **73**, 354-360.(2012)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Smoking, current	Higher amount smoked → earlier menopause	Highest quartile of pack-years vs never smoker, HR=1.52 (95% CI 1.30,1.79) (Q3 and Q4 significant) p<0.0048 for trend.	Multivariate Cox PH including parity, age at menarche, oral contraceptive use, unilateral oophorectomy, smoking status, education, vigorous physical activity, and BMI	Black Women's Health Study, USA	17,070	Aged 35-55 in 1995	Palmer, Rosenberg et al. Onset of natural menopause in African American women. *Am J Public Health*. **93**, 299-306.(2003)
Smoking, current	Current smoking → earlier menopause	Current smoker HR=1.43 (95% CI 1.24-1.66) vs never	Multivariate Cox PH including parity, age at menarche, oral contraceptive use, unilateral oophorectomy, smoking status, education, vigorous physical activity, and BMI	Black Women's Health Study, USA	17,070	Aged 35-55 in 1995	Palmer, Rosenberg et al. Onset of natural menopause in African American women. *Am J Public Health*. **93**, 299-306.(2003)
Smoking, current	Current smoking → earlier menopause	Current smokers 0.4 years earlier than never, p=0.05	ANCOVA - adjusted	Women visiting GPs in Italy in 1997	16,916	Aged 44-60 in 1997	Amigoni, Morelli et al. Cross-sectional study of determinants of menopausal age and hormone replacement therapy use in Italian women. *Climacteric*. **3**, 25-32.(2000)
Smoking, current	Smoking 10+ cigarettes per day → earlier menopause	Decrease in age at menopause by 1.3 years for 10+ cigarettes per day	Logisitic regression	Australian	15,464	(not stated in abstract)	Adena and Gallagher. Cigarette smoking and the age at menopause. *Ann Hum Biol*. **9**, 121-130.(1982)
Smoking, current	Higher amount smoked per day → earlier menopause	10-19 cigarettes per day vs none, HR=1.70 (95% CI 1.44,2.00), 20+ cigarettes per day HR=1.63 (95% CI 1.40,1.89)	Cox PH, adjusted for smoking, education, marital status, heart disease, parity, race/ethnicity, employment, oral contraceptives	Study of Women's Health Across the Nation (SWAN), USA	14,620	Aged 40-55 in 1995-1997	Gold, Bromberger et al. Factors associated with age at natural menopause in a multiethnic sample of midlife women. *American Journal of Epidemiology*. **153**, 865 - 874.(2001)
Smoking, current	Higher amount smoked per day → earlier menopause	20 or more cigarettes vs no smoking, HR=1.39 (95% CI 1.26,1.54)	Multivariate Cox PH, adjusted for age, population, education, marital status, smoking, BMI, physical activity, alcohol consumption, supplementation with vitamins and minerals, hormonal contraceptives, HRT.	Health, Alcohol and Psychosocial factors in Eastern Europe (HAPIEE), Russia, Poland, Czech Republic	12,676	Aged 45-69 in 2002-2005	Stepaniak, Szafraniec et al. Age at natural menopause in three central and eastern European urban populations: the HAPIEE study. *Maturitas*. **75**, 87-93.(2013)
Smoking, current	Smoking 30+ pack-years → earlier menopause	30+ pack-years smoking vs never smoked, OR=1.87 (95% CI 1.67,2.04)	Cases menopausal before 47 years, controls menopause at 47+ years. Kaplan-Meier, Cox PH, logistic regression. Adjusted for parity and weight.	Survey of women in Massachusetts, USA	10,606	Aged 45-54 years in 1989-1992	Cramer, Harlow et al. Cross-sectional and case-controlled analyses of the association between smoking and early menopause. *Maturitas*. **22**, 79 - 87.(1995)
Smoking, current	Current smoking → earlier menopause	Current smoker vs never HR=1.36 (95% CI 1.27,1.45), p<0.001	Cox PH, unadjusted. Log-rank test, unadjusted.	DOM-3 breast screening programme in Utrecht, The Netherlands	8,701	1932-1941	de Vries, den Tonkelaar et al. Oral contraceptive use in relation to age at menopause in the DOM cohort. *Hum Reprod*. **16**, 1657-1662.(2001)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Smoking, current	Current smoking → earlier menopause	Current smokers -1.74 years compared with non-smokers, p<0.01	t-test	White women in Massachusetts, USA	7,828	Aged 45-55 years	McKinlay, Bifano et al. Smoking and age at menopause in women. *Ann Intern Med*. **103**, 350-356.(1985)
Smoking, current	Current smoking → earlier menopause	Current smoking HR=1.29 (95% CI 1.14-1.46) vs never	Cox PH, adjusted for educational level, age at menarche, menstrual cycle length, oral contraceptives, live births, smoking status, and self-reported health status.	WOMID, Poland	7,183	Aged 35-65 in 2000-2004	Kaczmarek. The timing of natural menopause in Poland and associated factors. *Maturitas*. **57**, 139-153.(2007)
Smoking, current	Current smoking → earlier menopause	Smoker vs non-smoker, HR=1.19 (95% CI 1.06,1.32), p=0.018 for log-rank.	Log-rank. Cox PH adjusted.	Australian Twin Registry	5,961	Aged 17-88 in 1980-82, or aged 50+ in 1993-1995	Do, Treloar et al. Predictive factors of age at menopause in a large Australian twin study. *Hum Biol*. **70**, 1073-1091.(1998)
Smoking, current	Current smoking → earlier menopause	Current smoker vs never, HR=1.585 (95% CI 1.273,1.975)	Cox PH, adjusted for country	European Respiratory Health Survey (Spain, France, Belgium, Switzerland, UK, Norway, Sweden, Iceland, and Estonia) and the Swiss Air Pollution and Lung Disease in Adults Cohort	5,288	Aged 30-60 1998-2002	Dratva, Gomez Real et al. Is age at menopause increasing across Europe? Results on age at menopause and determinants from two population-based studies. *Menopause*. **16**, 385-394.(2009)
Smoking, current	Current smoking → earlier menopause	Smoker vs no second hand smoke exposure, OR=12.34 (95% CI 3.03,50.21) in blacks, OR=6.8 (95% CI 1.92,24.11) in Hispanic. Smoker vs passive smoker, OR=1.87 (95% CI 1.08,3.24) in whites.	Logistic regression, odds of post-menopausal vs pre-menopausal in women aged 25-50 years. Analysis in whites, blacks, hispanic.	National Health and Nutrition Examination Survey (NHANES), USA	5,029	Aged 25+ years in 1988-1994	Fleming, Levis et al. Earlier age at menopause, work, and tobacco smoke exposure. *Menopause*. **15**, 1103-1108.(2008)
Smoking, current	Current smoking → earlier menopause	Current smoker HR=1.40 (95% CI 1.13,1.58)	Cox PH adjusted for age.	European prospective investigation into cancer and nutrition (EPIC) - Heidelberg, Germany	4,807	Aged 35-65 in 1994-1998	Nagel, Altenburg et al. Reproductive and dietary determinants of the age at menopause in EPIC-Heidelberg. *Maturitas*. **52**, 337-347.(2005)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Smoking, current	Higher amount smoked → earlier menopause	21+ cigarettes per day vs 0, HR=1.36 (95% CI 1.06,1.69)	Cox PH, mutivariate model of age, parity, religion, BMI, cigarettes per day.	New York University Women Study, USA	4,694	Mean age 42.8 years in 1985-1991	Kato, Toniolo et al. Prospective study of factors influencing the onset of natural menopause.[see comment]. *Journal of Clinical Epidemiology*. **51**, 1271 - 1276.(1998)
Smoking, current	Current smoking → earlier menopause	Smokers 50.4 years vs non-smokers 50.9 years (p=0.01)	ANCOVA - adjusted for cohort of birth, BMI, smoking, age at menarche, number of births	Italian Climacteric Research Group Study (ICARUS) - prospective study of effect of menopause on womens' health	4,300	Aged >=55 in 1997	Meschia, Pansini et al. Determinants of age at menopause in Italy: results from a large cross-sectional study. *Maturitas*. **34**, 119-125.(2000)
Smoking, current	Current smoking → earlier menopause	Smoking vs non-smoker, HR=1.14 (95% CI 1.03,1.27)	Cox PH	Women from 7 cities in Latin America and the Caribbean	4,056	Aged 60-79 years	Velez, Alvarado et al. Life course socioeconomic adversity and age at natural menopause in women from Latin America and the Caribbean. *Menopause*. **17**, 552-559.(2010)
Smoking, current	Higher amount smoked → earlier menopause	0.48 for early vs 0.34 for late (Four categories of cigarettes per day: 0=0; 1= <10; 2=10 to 20; and 3= >20.) p<0.000	Chi-squared, or t-test. Not adjusted. Early menopause (<49 years) vs. late menopause (≥53 years).	Doorlopend Onderz oek Morbidi teit/Mortaliteit [DOM], The Netherlands	3,756	1911-1925	van Noord, Dubas et al. Age at natural menopause in a population-based screening cohort: the role of menarche, fecundity, and lifestyle factors. *Fertil Steril*. **68**, 95-102.(1997)
Smoking, current	Current smoking → earlier menopause	Current smoking vs non-smoker, beta=-0.08, p=0.00	Multiple linear regression, including age, height, weight, BMI, age at menarche, number of siblings, birth order, age at marriage, fecundity, age at first, childbirth, parity, smoking status, past smoking behavior, current amount of smoking, SES, oral contraceptive use	Doorlopend Onderz oek Morbidi teit/Mortaliteit [DOM], The Netherlands	3,756	1911-1925	van Noord, Dubas et al. Age at natural menopause in a population-based screening cohort: the role of menarche, fecundity, and lifestyle factors. *Fertil Steril*. **68**, 95-102.(1997)
Smoking, current	Current smoking → earlier menopause	Current smoking, OR=2.2 (95% CI 1.7,3.0)	Odds of being post-menopausal vs premenopausal, age adjusted	US 1999 National Health Interview Survey (NHIS)	3,307	1945-1959	Brett and Cooper. Associations with menopause and menopausal transition in a nationally representative US sample. *Maturitas*. **45**, 89 - 97.(2003)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Smoking, current	Smoking → earlier menopause	Higher % of never smokers in menopause<40 and ≥50, p=0.004 for association.	Chi-squared test against age at menopause in 4 categories (<40, 40-44, 45-49, ≥50)	National Health and Nutrition Examination Survey (NHANES), USA	3,191	Aged 25-74 years in 1971-1975	Cooper and Sandler. Age at natural menopause and mortality. *Ann Epidemiol*. **8**, 229-235.(1998)
Smoking, current	Current smoking → earlier menopause	Current smoking HR=1.30 (95% CI 1.003,1.682) in women with menopause <52 years	Cox PH analysis in women with menopause <52 years and 52+ years, adjusted for smoking status, parity, age at menarche, oral contraceptive, education, BMI, physical activity, language region	SAPALDIA Swiss cohort study on Air Pollution and Lung Diseases in Adults (SAPALDIA)	3,119	Aged 18-60 years in 1992	Dratva, Zemp et al. Variability of reproductive history across the Swiss SAPALDIA cohort--patterns and main determinants. *Ann Hum Biol*. **34**, 437-453.(2007)
Smoking, current	Higher amount smoked per day → earlier menopause	Highest dose (>10 cigarettes per day) vs 0 per day, OR=1.93 (95% CI 1.12,3.30)	Logistic regression, case was menopause at <45 years, adjusted for education.	Oslo Health Study, Norway	2,123	1940-41 (collected at age 59-60 years)	Mikkelsen, Graff-Iversen et al. Early menopause, association with tobacco smoking, coffee consumption and other lifestyle factors: a cross-sectional study. *BMC Public Health*. **7**, 149.(2007)
Smoking, current	Increased amount smoked during life → earlier menopause	For high exposure vs low exposure (cigarettes per year × time smoked), OR=1.79 (1.06,3.02)	Logistic regression, case was menopause at <45 years, adjusted for education.	Oslo Health Study, Norway	2,123	1940-41 (collected at age 59-60 years)	Mikkelsen, Graff-Iversen et al. Early menopause, association with tobacco smoking, coffee consumption and other lifestyle factors: a cross-sectional study. *BMC Public Health*. **7**, 149.(2007)
Smoking, current	Current smoking → earlier menopause	Current smoking OR=1.59 (95% CI 1.11,2.28)	Logistic regression, case was menopause at <45 years, adjusted for smoking, alcohol, coffee and education.	Oslo Health Study, Norway	2,123	1940-41 (collected at age 59-60 years)	Mikkelsen, Graff-Iversen et al. Early menopause, association with tobacco smoking, coffee consumption and other lifestyle factors: a cross-sectional study. *BMC Public Health*. **7**, 149.(2007)
Smoking, current	Current smoking → earlier menopause	Smoking HR=1.371 (95% CI 1.093,1.720)	Cox PH. Adjusted for episodic weight loss of more than 5kg.	Isparta Menopause and Health Study, Turkey (hospital based study)	1,106	Not known	Aydin. Determinants of age at natural menopause in the Isparta Menopause and Health Study: premenopausal body mass index gain rate and episodic weight loss. *Menopause*. **17**, 494-505.(2010)
Smoking, current	Smoking → earlier menopause	Smokers 48.6 years vs 49.5 years for never smokers, p=0.01	t-test, linear trend	Patients at hospitals in Milan, Italy	863	Aged 55-74 in 1983-1989	Parazzini, Negri et al. Reproductive and general lifestyle determinants of age at menopause. *Maturitas*. **15**, 141-149.(1992)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Smoking, current	Higher amount smoked → earlier menopause	Never smoked 49.4 years vs ex-smokers 49.2 years, current smoker 1-14 cigarettes 48.0 years, current smoker 15+ cigarettes per day 47.6 years; p < 0.02 for each category compared with never. Results not changed when stratified.	Comparison of mean natural menopause age in never smoked, previous, current. Analaysis stratified by parity, ponderal index, geographic region.	Survey of patients at 7 hospitals in US, 1 in Canada, 2 in Israel.	656	Aged 60-69 in 1976-1978	Kaufman, Slone et al. Cigarette smoking and age at natural menopause. *Am J Public Health*. **70**, 420-422.(1980)
Smoking, current	Current smoking → earlier menopause	Current smokers vs never, risk ratio=1.3 (95% CI 1.0,1.7)	Cox PH	Menstruation and Reproductive History Study (Minnesota, USA)	543	~1920	Cooper, Sandler et al. Active and passive smoking and the occurrence of natural menopause. *Epidemiology*. **10**, 771 - 773.(1999)
Smoking, current	Current smoking → earlier menopause	Current smoker vs never, 1.9 years earlier (95% CI -3.5,-0.2)	Parametric logistic survival analysis. Multi-variate model including pregnancy, alcohol, caffeine and smoking	Case-control study of spontaneous abortion, New York, USA	494	1933-1942	Kinney, Kline et al. Alcohol, caffeine and smoking in relation to age at menopause. *Maturitas*. **54**, 27-38.(2006)
Smoking, current	Current smoking → earlier menopause	Smokers (47.1 years) vs non-smokers (49.4 years), p<0.00001	t-test, chi-squared test, unadjusted	Menopause Centre, Civitanova Hospital, Italy	350	Patients attending hospital 1997-2001	Di Prospero, Luzi et al. Cigarette smoking damages women's reproductive life. *Reprod Biomed Online*. **8**, 246-247.(2004)
Smoking, ever	Ever smoker → earlier menopause	Ever smoker vs never, HR=1.20 (95% CI 1.12,1.29)	Multivariate Cox PH, endpoints menopause, early menopause (<45 years), premature ovarian failure (<40 years). Adjusted for age at menarche, number of deliveries, current BMI, cycle regularity, unilateral oophorectomy, oral contraceptives, ever smoker before menopause and birth year decade.	Japan Nurses' Health Study (JNHS)	24,152	Aged 40-59 years 2001-2007	Yasui, Hayashi et al. Factors associated with premature ovarian failure, early menopause and earlier onset of menopause in Japanese women. *Maturitas*. **72**, 249-255.(2012)
Smoking, ever	Ever smoking → earlier menopause	Ever smoked vs non-smokers, OR=1.31 (95% CI 1.21,1.42)	Cases menopausal before 47 years, controls menopause at 47+ years. Kaplan-Meier, Cox PH, logistic regression. Adjusted for parity and weight.	Survey of women in Massachusetts, USA	10,606		Cramer, Harlow et al. Cross-sectional and case-controlled analyses of the association between smoking and early menopause. *Maturitas*. **22**, 79 - 87.(1995)
Smoking, ever	Ever smoker → earlier menopause	29.7% smokers for early vs 22.6% for late, p<0.000	Chi-squared, or t-test. Not adjusted. Early menopause (<49 years) vs. late menopause (≥53 years)	Doorlopend Onderz oek Morbidi teit/Mortaliteit [DOM], The Netherlands	3,756	1911-1925	van Noord, Dubas et al. Age at natural menopause in a population-based screening cohort: the role of menarche, fecundity, and lifestyle factors. *Fertil Steril*. **68**, 95-102.(1997)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Smoking, passive	Passive smoking → earlier menopause	Second hand smoke exposure vs no exposure, OR=6.65 (95% CI 1.45,30.40) in blacks, OR=19.08 (95% CI 5.96,61.07) in Hispanic.	Logistic regression, odds of post-menopausal vs pre-menopausal in women aged 25-50 years. Analysis in whites, blacks, hispanic.	National Health and Nutrition Examination Survey (NHANES), USA	5,029	Aged 25+ years in 1988-1994	Fleming, Levis et al. Earlier age at menopause, work, and tobacco smoke exposure. *Menopause*. **15**, 1103-1108.(2008)
Smoking, previous	Ex-smoker → earlier menopause	Never as reference; for smoker at 45, ≤10 cigs per day HR=1.10 (95% CI 1.07,1.13) for >10 cigs per day HR=1.21 (95% CI 1.18,1.24).	Age at menopause collected as categories (<45, 45-49, 50-54, ≥55). Mulitvariable Cox PH adjusted for ethnicity, smoking, menarche age, parity, BMI	Multiethnic Cohort Study, USA (non-Latina Whites, Japanese Americans, African Americans, Native Hawai'ians, and Latinas).	95,704	Aged 45-74 years in 1993-1996	Henderson, Bernstein et al. Predictors of the timing of natural menopause in the Multiethnic Cohort Study. *Am J Epidemiol*. **167**, 1287-1294.(2008)
Smoking, previous	Higher amount smoked previously → earlier menopause	Never as reference; for ex-smoker at 45, ≤10 cigs per day HR=1.05 (95% CI 1.09,1.09); >10 cigs per day HR=1.16 (1.11,1.22)	Age at menopause collected as categories (<45, 45-49, 50-54, ≥55). Mulitvariable Cox PH adjusted for ethnicity, smoking, menarche age, parity, BMI	Multiethnic Cohort Study, USA (non-Latina Whites, Japanese Americans, African Americans, Native Hawai'ians, and Latinas).	95,704	Aged 45-74 years in 1993-1996	Henderson, Bernstein et al. Predictors of the timing of natural menopause in the Multiethnic Cohort Study. *Am J Epidemiol*. **167**, 1287-1294.(2008)
Smoking, previous	Previous smoking → later menopause	Ex-smoker vs never, HR=0.96 (95% CI 0.93,0.99)	Competing risks Cox PH adjusted for age, smoking status, parity, BMI at 40 years.	Breakthrough Generations Study, UK	50,678	Aged 40-98 in 2003-2011	Morris, Jones et al. Body mass index, exercise, and other lifestyle factors in relation to age at natural menopause: analyses from the breakthrough generations study. *Am J Epidemiol*. **175**, 998-1005.(2012)
Smoking, previous	Previous smoker → earlier menopause	Past smoker 48.3 years vs 48.7 years for never (p<0.005)	Multiple linear regression of geographical region, race,age, parity, smoking, region, income, education, physical activity, and history of CVD	REasons for Geographic And Racial Differences in Stroke and Myocardial Infarction (REGARDS), USA	22,484	Aged >=45 in 2003-2007)	McKnight, Wellons et al. Racial and regional differences in age at menopause in the United States: findings from the REasons for Geographic And Racial Differences in Stroke (REGARDS) study. *Am J Obstet Gynecol*. **205**, 353.e351-358.(2011)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Smoking, previous	Previous smoker → earlier menopause	Past smoker vs never HR=1.21 (95% CI 1.06,1.38)	Multivariate Cox PH including parity, age at menarche, oral contraceptive use, unilateral oophorectomy, smoking status, education, vigorous physical activity, and BMI	Black Women's Health Study, USA	17,070	Aged 35-55 in 1995	Palmer, Rosenberg et al. Onset of natural menopause in African American women. *Am J Public Health*. **93**, 299-306.(2003)
Smoking, previous	Previous smoker → earlier menopause	Past smoker vs never, HR=1.13 (95% CI 1.07,1.21)	Cox PH, adjusted for educational level, age at menarche, menstrual cycle length, oral contraceptives, live births, smoking status, and self-reported health status.	WOMID, Poland	7,183	Aged 35-65 in 2000-2004	Kaczmarek. The timing of natural menopause in Poland and associated factors. *Maturitas*. **57**, 139-153.(2007)
Smoking, previous	Higher amount smoked previously → earlier menopause	Three categories of cigarettes per day: 0=0; 1= ≤20; 2= >20. 0.34 for early vs 0.27 for late.	Chi-squared, or t-test. Not adjusted. Early menopause (<49 years) vs. late menopause (≥53 years).	Doorlopend Onderz oek Morbidi teit/Mortaliteit [DOM], The Netherlands	3,756	1911-1925	van Noord, Dubas et al. Age at natural menopause in a population-based screening cohort: the role of menarche, fecundity, and lifestyle factors. *Fertil Steril*. **68**, 95-102.(1997)
Smoking, previous	Stopped smoking >10 years before menopause → later menopause compared with current smokers	For stopped >10 years vs current smokers, OR=0.13 (95% CI 0.05,0.33)	Logistic regression, case was menopause at <45 years, adjusted for education.	Oslo Health Study, Norway	2,123	1940-41 (collected at age 59-60 years)	Mikkelsen, Graff-Iversen et al. Early menopause, association with tobacco smoking, coffee consumption and other lifestyle factors: a cross-sectional study. *BMC Public Health*. **7**, 149.(2007)
Social participation	Higher social participation → later menopause	High social participation vs low, OR=0.60 (95% CI 0.39,0.98)	Logistic regression, case was menopause at <45 years, adjusted for smoking, alcohol, coffee and education.	Oslo Health Study, Norway	2,123	1940-41 (collected at age 59-60 years)	Mikkelsen, Graff-Iversen et al. Early menopause, association with tobacco smoking, coffee consumption and other lifestyle factors: a cross-sectional study. *BMC Public Health*. **7**, 149.(2007)
Weight	Higher adult weight → later menopause	Weight at 40 years, 60-64.9 kg as reference. <55kg HR=1.06 (95% CI 1.02,1.09), for 70+ kg HR=0.90 (95% CI 0.87,0.93), p<0.001 for trend	Competing risks Cox PH adjusted for age, smoking status, parity, BMI at 40 years.	Breakthrough Generations Study, UK	50,678	Aged 40-98 in 2003-2011	Morris, Jones et al. Body mass index, exercise, and other lifestyle factors in relation to age at natural menopause: analyses from the breakthrough generations study. *Am J Epidemiol*. **175**, 998-1005.(2012)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Weight	Higher weight age 20 → later menopause	Weight ≥54kg vs <45kg, beta=0.30 p<0.01	Multi-variable linear regression, including age, education, occupation, income, marital status, current employment, energy intake, weight gain between age 20 and 50 years, cigarette smoking (never/ever), and leisure-time physical activity patterns in adolescence and adulthood.	Shanghai Women's Health Study	33,054	Aged 40-70 years in 1997-2000	Dorjgochoo, Kallianpur et al. Dietary and lifestyle predictors of age at natural menopause and reproductive span in the Shanghai Women's Health Study. *Menopause*. **15**, 924-933.(2008)
Weight	Higher weight → later menopause	Increasing baseline weight interquartile range, HR = 0.92 (95% CI 0.84,0.996)	Cox PH adjusted for race/ethnicity, baseline smoking, time-varying smoking, health baseline, educational level, use of oral contraceptives at baseline, alcohol at baseline, alcohol change since baseline, current employment, physical activity, percentile of baseline weight. 10 years follow-up	Study of Women's Health Across the Nation (SWAN), USA	3,302	Aged 40-55 in 1995-1997	Gold, Crawford et al. Factors related to age at natural menopause: longitudinal analyses from SWAN. *Am J Epidemiol*. **178**, 70-83.(2013)
Weight, change	Higher weight gain from 20-40 years → later menopause	Weight change 20-40 years. Gained 14+ kg vs no change, HR=0.93 (95% CI 0.87,0.98); p<0.012 for trend	Competing risks Cox PH adjusted for age, smoking status, parity, BMI at 40 years.	Breakthrough Generations Study, UK	50,678	Aged 40-98 in 2003-2011	Morris, Jones et al. Body mass index, exercise, and other lifestyle factors in relation to age at natural menopause: analyses from the breakthrough generations study. *Am J Epidemiol*. **175**, 998-1005.(2012)
Weight, change	Weight gain → later menopause	Weight gain 20-50 years, ≥12.5 kg vs <2.0 kg, beta=0.44, p<0.01.	Multi-variable linear regression, including age, education, occupation, income, marital status, current employment, energy intake, weight gain between age 20 and 50 years, cigarette smoking (never/ever), and leisure-time physical activity patterns in adolescence and adulthood.	Shanghai Women's Health Study	33,054	Aged 40-70 years in 1997-2000	Dorjgochoo, Kallianpur et al. Dietary and lifestyle predictors of age at natural menopause and reproductive span in the Shanghai Women's Health Study. *Menopause*. **15**, 924-933.(2008)
Weight, change	Increased weight premenopause → later menopause; decreased weight premenopause → earlier menopause	Per 5% increase in weight, 7.15 years (95% CI -12.00,-2.33), per 5% decrease in weight, -3.54 years (95% CI -6.2,-0.88)	Linear regression adjusted for smoking	Framingham Heart Study cohort	695	Aged 29-62 years in 1948	Kok, van Asselt et al. Heart disease risk determines menopausal age rather than the reverse. *J Am Coll Cardiol*. **47**, 1976-1983.(2006)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Year of birth	Earlier year of birth → earlier menopause	Year of birth 1960-69 vs year of birth <1950, HR=0.632 (95% CI 0.410,0.972). For EM, 1950-59 vs <1950, HR=0.745 (95% CI 0.562,0.988)	Multivariate Cox PH, endpoints menopause, early menopause (<45 years), premature ovarian failure (<40 years). Adjusted for age at menarche, number of deliveries, current BMI, cycle regularity, unilateral oophorectomy, oral contraceptives, ever smoker before menopause and birth year decade.	Japan Nurses' Health Study (JNHS)	24,152	Aged 40-59 years 2001-2007	Yasui, Hayashi et al. Factors associated with premature ovarian failure, early menopause and earlier onset of menopause in Japanese women. *Maturitas*. **72**, 249-255.(2012)
Year of birth	Earlier year of birth → earlier menopause	1932-34 vs 1935-1938 vs 1939-1941, p<0.001 for association	Log-rank test, unadjusted	DOM-3 breast screening programme in Utrecht, The Netherlands	8,701	1932-1941	de Vries, den Tonkelaar et al. Oral contraceptive use in relation to age at menopause in the DOM cohort. *Hum Reprod*. **16**, 1657-1662.(2001)
Year of birth	Earlier year of birth → earlier menopause	Increasing year of birth, HR=0.98 (95% CI 0.97,0.98), p<0.001 for log-rank.	Log-rank. Cox PH adjusted.	Australian Twin Registry	5,961	Aged 17-88 in 1980-82, or aged 50+ in 1993-1995	Do, Treloar et al. Predictive factors of age at menopause in a large Australian twin study. *Hum Biol*. **70**, 1073-1091.(1998)
Year of birth	Earlier year of birth → earlier menopause	Per year increase, HR=0.913 (95% CI 0.878, 0.950) (in SAPALDIA only)	Cox PH, adjusted for country	European Respiratory Health Survey (Spain, France, Belgium, Switzerland, UK, Norway, Sweden, Iceland, and Estonia) and the Swiss Air Pollution and Lung Disease in Adults Cohort	5,288	Aged 30-60 1998-2002	Dratva, Gomez Real et al. Is age at menopause increasing across Europe? Results on age at menopause and determinants from two population-based studies. *Menopause*. **16**, 385-394.(2009)
Year of birth	Earlier year of birth → earlier menopause	17-month increase from 1915-1939 (49.1 vs. 50.5 years; p=0.001)	Linear regression adjusted for state, current smoking, education, parity, age at last birth, height, and BMI, in women who never used HRT	Collaborative Breast Cancer Study (New Hampshire, Massachusetts, Wisconsin), USA	4,767	1910-1969	Nichols, Trentham-Dietz et al. From menarche to menopause: trends among US Women born from 1912 to 1969. *Am J Epidemiol*. **164**, 1003-1011.(2006)

Variable	Effect	Effect size and significance	Analysis type	Study details	n	Birth year/Age	Reference
Year of birth	Earlier year of birth → earlier menopause	For year of birth 1911-1925, beta=0.016, p=0.002	Linear regresission in women not using oral contraceptives	Doorlopend Onderz oek Morbidi teit/Mortaliteit [DOM], The Netherlands	3,756	1911-1925	van Noord, Dubas et al. Age at natural menopause in a population-based screening cohort: the role of menarche, fecundity, and lifestyle factors. *Fertil Steril*. **68**, 95-102.(1997)
Year of birth	Earlier year of birth → earlier menopause	Increase of 0.1 years per birth year (p<0.0001)	Adjusted for SES, smoking, oral contraceptives, HRT	Prospective study, Gothenburg, Sweden	1,017	1930, 1922, 1918, 1914, 1908	Rodstrom, Bengtsson et al. Evidence for a secular trend in menopausal age: a population study of women in Gothenburg. *Menopause*. **10**, 538-543.(2003)

Appendix 3. Genes associated with age at menopause in human studies.

ANM=age at natural menopause; POI=primary ovarian insufficiency.

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
ABAT	ANM	4-aminobutyrate aminotransferase, mitochondrial	Catabolism of gamma-aminobutyric acid (GABA), a neurotransmitter in the central nervous system.	16p13.2	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs9039, effect -0.12 (0.02), p=3.3E-08	1
ACSL6	POI	Acyl-CoA synthetase long-chain family member 6	Activation of long-chain fatty acids, role in fatty acid metabolism in brain.	5q31	Kang, et al. 2009: 24 women with POF, 24 matched controls, 6 SNPs in a haplotype significantly associated with POF	2
ADAMTS19	POI	A disintegrin and metalloproteinase with thrombospondin motifs 19	Releases extracellular domains of transmembrane proteins by proteolytic cleavage. Thought to play a role in gonad formation and function.	5q31	Knauff, et al. 2009: GWAS on Caucasians, 99 unrelated idiopathic POF vs 235 controls. Suggestive association of rs246246, p=6.0E-07, but replication in an independent Dutch cohort (60 POF and 90 controls) did not confirm - joint p=4.1E-05. ADAMTS19 known to be up-regulated in female mouse gonads during sexual differentiation. Pyun, et al. 2013: Analysed interactions between SNPs and diplotypes in IGF2R and ADAMTS19. 120 Korean patients with POF and 152 controls, plus an additional 1641 female controls. 4 combinations of SNPs in IGF2R and ADAMTS19 were significant (p<0.0071).	3, 4
AFF2	POI	AF4/FMR2 family member 2; FMR2, fragile X mental retardation 2; FRAXE	Putative transcriptional activator. A repeat polymorphism in folate-sensitive fragile X E locus on chromosome X results in silencing of this gene causing Fragile X E syndrome, a form of nonsyndromic X-linked mental retardation.	Xq28	Murray, et al. 1998: Screened FMR1 and FMR2 in 147 women with idiopathic POF. Excess of small alleles with fewer than 11 repeats, and one small deletion. Murray, et al. 1999: 209 females with POF, 3 females (1.5% of cases) with cryptic deletions in FMR2.	5, 6
ALOX12	POI	Arachidonate 12-lipoxygenase	Lipid metabolism, generates bioactive lipid mediators that regulate processes such as platelet activation, angiogenesis, cell migration, proliferation.	17p13.1	Liu, et al. 2010: Genotyped 6 SNPs of ALOX12 in 210 white women for association with ANM. rs9904779 and rs434473 (encodes a replacement of asparagine by serine) significantly associated with ANM (P = 0.022 and 0.033, respectively), ANM 1.3- to 1.5-year earlier for minor alleles.	7

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
AMH	POI	Anti-Mullerian hormone	Part of transforming growth factor-beta superfamily. Involved in primary follicle formation, required for male sexual differentiation.	19p13.3-p13.2	Alvaro Mercadal, et al. 2015: Sequenced coding sequence and exon-intron junction of AMH gene. Functional assays. 55 POI patients vs 197 ethnically matched controls. 16 variants in AMH: 6 missense. 1 unknown (R444H), hertozygous, Caucasian patient, no stimulation of AMHR2 receptor. Rare mutation G264R, heterozygous, African patient, no stimulation of AMHR2 receptor. D288E, heterozygous, Caucasian patient, reduced stimulation of AMHR2 receptor.	8
AMHR2	ANM	Anti-Mullerian hormone type-2 receptor	Receptor for AMH.	12q13	Kevenaar, et al. 2007: 2 large population-based cohorts of Dutch post-menopausal women (n = 2381 and n = 248). Observed association of AMHR2 -482 A > G (rs2002555) polymorphism with natural age at menopause. Voorhuis, et al. 2011: Analysed 23 SNPs in 5 genes: AMH, AMHR2, BMP15, FOXL2, GDF9 in 3616 Dutch women. rs2002555 and rs11170547 in AMHR2 gene associated with ANM in interaction with parity. In parous women, rs2002555 G/G carriers menopause 1 yr later compared with A/A carriers (P=0.01), each T allele of rs11170547 associated with a 0.41-yr later onset of menopause (P=0.01). Chen, et al. 2014: Replication GWAS in 6510 African American women, rs2002555, effect 0.332 years, p=0.0062	9, 10, 11
ANKK1	ANM	Ankyrin repeat and kinase domain containing 1	Signal transduction, member of Ser/Thr protein kinase family.	11q23.2	He, et al. 2010: Evaluation of 278 candidate genes in 12,723 women (Nurses' Health Study and Women's Genome Health Study studies). P-value adjusted for LD and number of tests. rs6279, effect 0.17 years, p=0.044	12
APEX1	ANM	APEX nuclease (multifunctional DNA repair enzyme) 1	Cellular response to oxidative stress via DNA repair and regulation of transcription factors.	14q11.2	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs1713460, effect -0.14 (0.02), p=2.4E-10	1
APOE	ANM	Apolipoprotein E	Mediates lipoprotein particle binding, internalization, and catabolism. ApoE ε4 linked to Alzheimer's disease, atherosclerosis.	19q13.31	Tempfer, et al. 2005: Analysed 8 SNPs in 6 genes, 728 white European women. APOE-2 Arg158Cys associated with earlier menopause (p=0.03). Meng, et al. 2012: ApoE genotype associated with ANM (P=0.010). Compared ApoE ε3/4 females had ANM 1.8-years later than ApoE ε3/3 (P=0.002). ANM delayed in ApoE ε4 carriers (P=0.023).	13, 14
APTX	ANM	Aprataxin	DNA-binding protein involved in DNA break repair and base excision repair.	9p13.3	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs4879656, effect -0.12 (0.02), p=2.0E-08	1

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
AR	POI	Androgen receptor	Steroid hormone receptor. Ligand-activated transcription factor.	Xq11.2-q12	Laisk, et al. 2010: 26 women with POF, 32 women with PCOS, 79 controls. Analysed length of AR CAG repeat and X chromosome inactivation. POF patients had shorter AR-CAG microsatellites than controls (p=0.012; p=0.004). Polymorphic CAG repeat in exon 1 of AR is translated into a polyglutamine tract - length alters the conformation of the N-terminal transcription activation domain of the androgen receptor. Panda, et al. 2011: 133 cases of POF, 63 primary amenorrhea cases, 200 controls. Mutational analysis of AR gene. 4 novel mutations c.636G>A, c.1885+9C>A, c.1948A>G, c.1972C>A. 2 previously reported mutations, c.639G>A, c.2319-78T>G.	15, 16
ARHGEF7	ANM	Rho guanine nucleotide exchange factor (GEF) 7	Functions in cell migration, attachment and cell spreading.	13q34	Stolk, et al. 2009: GWAS in 2,979 Europeans (Rotterdam, Twins UK studies). Rs7333181, effect 0.5 years, p=2.50E-08.	17
ASCL1	ANM	Achaete-scute homolog 1	Transcription factor. Role in early stages of development of CNS and peripheral nervous system.	4q35.1	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs6856693, effect -0.16 (0.02), p=9.8E-15	1
BCAR4	ANM	Breast cancer anti-oestrogen resistance 4 protein	Expressed in oocytes in cattle. Identified from screen of genes involved in tamoxifen resistance	16p13.13	Stolk, et al. 2012: HapMap imputed GWAS meta-analysis 38,968 women of European descent, with replication in up to 14,435 women. rs10852344, effect 0.168 years, p=1.01E−11. Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs10852344, effect -0.16 (0.02), p=1.3E-15	18, 1
BCKDHB	POI	Branched chain keto acid dehydrogenase E1, beta polypeptide	Catabolism of branched chain amino acids. Gene encodes E1 beta subunit - mutations in E1 cause maple syrup urine disease (MSUD), type 1B.	6q14.1	Kang, et al. 2008: Genotyping in 16 women with POF and 16 matched controls. 10 SNPs in haplotype block significantly associated with POF.	19
BMP15	ANM	Bone morphogenic protein 15	Oocyte-specific growth/differentiation factor, stimulates folliculogenesis and granulosa cell growth	Xp11.22	Voorhuis, et al. 2011: Analysed 23 SNPs in 5 genes: AMH, AMHR2, BMP15, FOXL2, GDF9 in 3616 Dutch women. rs6521896 in BMP15 effect= 0.41 years, P=0.007.	10

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
	POI	Bone morphognic protein 15	Oocyte-specific growth/differentiation factor, stimulates folliculogenesis and granulosa cell growth	Xp11.22	Di Pasquale, et al. 2006: 166 unrelated patients with idiopathic POF (25 primary amenorrhea, 141 secondary amenorrhea vs controls (95 women with menopause> 50 years; 86 women and 30 men from the general population), all Caucasian. Identified 4 heterozygous variants in 7 secondary amenorrhea patients (P<0.003 vs. controls) - previously reported p.Y235C mutation (one case), two missense alterations (p.R68W in one case, p.A180T in five). Dixit, et al. 2006: Analysis of the coding region of BMP15 in 133 women with POI, 60 with primary amenorrhoea, 9 with secondary amenorrhoea and 197 controls. 18 variants in BMP15 - 13 missense. 11 missense variants only in cases. Laissue, et al. 2006: Sequenced GDF9 and BMP15 in 203 POI patients and 54 controls. Heterozygous missense variant L148P at conserved position, not found in controls. Wang, et al. 2010: Analysis of coding regions of GDF9 and BMP15 in 100 Chinese POI patients. Novel missense mutations c.985C>T in BMP15 absent from controls and likely to have functional effect. Also SNP c.598C>T.	20, 21, 22, 23
BRCA1	ANM	Breast cancer type 1 susceptibility protein	Part of complex involved in double strand break repair, tumour suppressor.	17q21.31	Lin, et al. 2013: Compared age at menopause in BRCA1/2 carriers from registry (n=382) and non-registry women. In BRCA1/2 carriers, ANM earlier than non-breast cancer cases (50 vs 53 years, p-value<0.001) and HR (for menopause, adjusted for potential confounders)=3.98 (95% CI 2.87-5.53). Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs1799949, effect -0.14 (0.02), p=8.4E-11	24, 1
BRCA2	ANM	Breast cancer type 2 susceptibility protein	Part of complex involved in double strand break repair, binds RAD51 recombinase	13q12-q13	Lin, et al. 2013: Compared age at menopause in BRCA1/2 carriers from registry (n=382) and non-registry women. In BRCA1/2 carriers, ANM earlier than non-breast cancer cases (50 vs 53 years, p-value<0.001) and HR (for menopause, adjusted for potential confounders)=3.98 (95% CI 2.87-5.53).	24
BRE	ANM	Brain and reproductive organ-expressed (TNFRSF1A modulator); BRCA1/BRCA2-containing complex, subunit 4, BRCC4, BRCC45	Part of BRCA1-A complex, involved in double strand break damage repair.	2p23.3	Stolk, et al. 2012: HapMap imputed GWAS meta-analysis 38,968 women of European descent, with replication in up to 14,435 women. rs2303369, effect −0.175, p=2.25E−12 Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs704795, effect -0.16 (0.02), p=2.1E-15	18, 1

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
BRSK1	ANM	BR serine/threonine kinase 1	Polarization of neurons. Role in centrosome duplication via phosphorylation of gamma-tubulin. Part of UV-induced damage checkpoint response.	19q13.42	He, et al. 2009: GWAS in 9,112 Europeans (Nurses' Health Study, Women's Genome Health Study studies). 6 SNPs at this locus. rs1172822 had smallest p, effect -0.49 years, p=1.8E-19. Candidates: BRSK1, HSPBP1, SUV420H2. Stolk, et al. 2009: GWAS in 2,979 Europeans (Rotterdam, Twins UK studies). 4 SNPs at this locus. rs1172822 had smallest p, effect=-0.4 years, p=6.28E-11. Candidates: BRSK1, TMEM224, SUV420H2. Stolk, et al. 2012: GWAS meta-analysis in 38,968 Europeans, replication in 14,435. rs11668344, effect -0.4 years, p=1.45E10-59. Candidate: TMEM150B. Other genes: BRSK1, HSPBP1, COX6B2, LOC284417, IL11, SUV420H2 Chen, et al. 2012: Replication GWAS in 3468 Hispanic women, rs17782355, effect -1.4 years, p=0.0064 Shen, et al. 2013: Replicated rs7246479 in GWAS of 3,533 Chinese women. Effect= 0.49 years, p=3.75E-05. Replicated rs1172822. Effect= -0.6 years, p=6.64E-05. Chen, et al. 2014: Replication GWAS in 6510 African American women, rs11668344, effect -0.31 years, p=6.54E-04 Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs11668344, effect -0.41 (0.02), p=4.2E-84; rs2547274, effect -0.22 (0.04), p=2.7E-08; rs12461110, effect -0.15 (0.02), p=5.0E-14	1; 25, 17, 26, 27, 11
C16orf72	ANM	Chromosome 16 open reading frame 72	Unknown	16p13.2	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs9039, effect -0.12 (0.02), p=3.3E-08	1
CDK12	ANM	Cyclin-dependent kinase 12	Phosphorylates RNA polymerase II (POLR2A), regulates transcription elongation. Required for RNA splicing. Involved in regulation of MAP kinase activity.	17q12	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs2941505, effect -0.13 (0.02), p=1.9E-09	1
CDKN1B	POI	Cyclin-dependent kinase inhibitor 1B (p27, Kip1); KIP1, P27KIP1	Regulator of cell cycle, involved in G1 arrest.	12p13.1-p12	Ojeda, et al. 2011: Analysed gene in 87 Tunisian POI patients, 137 Tunisian and 126 Colombian controls. Novel heterozygous p.Ile119Thr mutation in one patient.	28

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
CENPU	ANM	Centromere protein U; MLF1 interacting protein", MLF1IP; CENP-50, CENP-U, KLIP1, PBIP1	Component of nucleosome-associated complex. Important for assembly of kinetochore proteins, mitotic progression and chromosome segregation.	4q35.1	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs6856693, effect -0.16 (0.02), p=9.8E-15	1
CHD7	ANM	Chromodomain-helicase-DNA-binding protein 9	Transcriptional coactivator for nuclear receptors, proposed to be a chromatin remodelling protein, DNA-dependent ATPase activity, binding A/T-rich DNA.	8q12.2	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs10957156, effect -0.14 (0.02), p=4.5E-09	1
CHEK2	ANM	Checkpoint kinase 2	Required for checkpoint-mediated cell cycle arrest, activation of DNA repair and apoptosis in response to the presence of DNA double-strand breaks..	22q12.1	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs5762534, effect -0.16 (0.03), p=6.1E-09	1
CITED2	POI	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2 ; MRG1	Transcriptional coactivator /corepressor. Role in sex determination and early gonad development, left-right patterning during embryogenesis, differentiation of adrenal cortex.	6q23.3	Fonseca, et al. 2012: Sequenced CITED2 in 139 women with POI and 290 controls. 5 synonymous and 3 nonsynonymous variants.7 novel. Nonsynonymous variant c.604C>A (p.Pro202Thr) found uniquely in 1 woman from the POF group - in silico analysis indicated a potential deleterious effect.	29
CLPP	POI	Caseinolytic mitochondrial matrix peptidase proteolytic subunit	Component of a mitochondrial ATP-dependent proteolytic complex.	19p13.3	Jenkinson, et al. 2013: Perrault syndrome is characterised by sensorineural hearing loss and ovarian failure. Linkage analysis, homozygosity mapping, and exome sequencing in 3 families with Perrault syndrome. Affected homozygous for: c.433A>C (p.Thr145Pro), c.440G>C (p.Cys147Ser), or splice-donor-site mutation, c.270+4A>G. Suggest that both missense substitutions alter structure of the CLPP barrel chamber that exposes unfolded proteins to proteolysis.	30

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
COX10	POI	COX10 haem A:farnesyltransferase cytochrome c oxidase assembly factor	Nuclear-encoded factor needed for assembly of cytochrome C oxidase, final enzyme in mitochondrial respiratory chain.	17p12	Pitceathly, et al. 2013: Adult patient with isolated COX deficiency mild clinical phenotype - myopathy; demyelinating neuropathy; premature ovarian failure; short stature; hearing loss; pigmentary maculopathy; and renal tubular dysfunction. Whole-exome sequencing: c.1007A>T; p.Asp336Val, previously associated with fatal infantile COX deficiency; novel, c.1015C>T; p.Arg339Trp. Both mutations demonstrated respiratory deficiency in yeast, confirming pathogenicity.	31
CPEB1	POI	Cytoplasmic polyadenylation element-binding protein 1	RNA-binding protein, regulates mRNA cytoplasmic polyadenylation and translation initiation during oocyte maturation, early development and at post-synapse sites of neurons.	15q25.2	McGuire, et al. 2011: 89 POF patients. Microdeletion causing haploinsufficiency for CPEB1.	32
CYP19A1	ANM	Cytochrome P450, family 19, subfamily A, polypeptide 1	Haem binding, oxido-reductase activity	15q21	He, et al. 2010: Evaluation of 278 candidate genes in 12,723 women (Nurses' Health Study and Women's Genome Health Study studies). P-value adjusted for LD and number of tests. Rs11856927, effect 0.19 years, p=0.023	12
	POI	Cytochrome P450, family 19, subfamily A, polypeptide 1	Haem binding, oxido-reductase activity	15q21	Kim, et al. 2011: 98 POF patients, 218 matched controls, investigated associations between SNPs in FSHR and CYP19A1 and POI. OR=5.42 (95% CI 1.96,14.98) for rs4646 (CA+AA) in the 3'UTR of CYP19A1 and missense FSHR SNP rs6166 (AG+GG). OR=5.47 (95% CI 2.03,14.75) for FSHR missense SNP rs6166 (AA) and rs4646-rs10046 CYP19A1 haplotype (C-T)+(C-C).	33
CYP1B1	ANM	Cytochrome P450, family 1, subfamily B, poly-peptide 1	Metabolism of pro-carcinogens and 17beta-oestradiol.	2p22.2	Hefler, et al. 2005: 1360 Caucasian women, CYP1B1-4 A>G, Asn453Ser, effect -0.8 years for heterozygous and homozygous for minor allele, p=0.004. Long, et al. 2006: 1958 women, genotyped four SNPs in CYP1B1, linear regression of menopause age adjusted for confounders. Arg48Gly 0.6 years later, p=0.02; Ala119Ser 0.9 years later, p=0.04; Leu432Val 1 year earlier (p=0.004). Butts, et al. 2014: 410 women from Penn Ovarian Aging study, European-Americans.CYP1B1*4 Asn452Ser, CYP1B1*3 Leu432Val. HR of menopause in smokers vs non-smokers. CYP1B1*3 (rs1056836) carriers: HR(adjusted)=2.26 (95% CI 1.4,3.67), no association in wildtype. HR was increased for heavy smokers.	34, 35, 36

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
CYP3A4	ANM	Cytochrome P450, family 3, subfamily A, polypeptide 4.	Metabolism of steroids and carcinogens.	7q22.1	Butts, et al. 2014: 410 women from Penn Ovarian Aging study, European-Americans. CYP3A4*1B (rs2740574) carriers: HR(adjusted)=15.1 (95% CI 3.31-69.2), and in wildtypes HR=1.59 (95% CI 1.03,2.44). HR was increased for heavy smokers.	36
DACH2	POI	Dachshund family transcription factor 2; dachshund homolog 2	Transcription factor, involved in regulation of organogenesis	Xq21.3	Prueitt, et al. 2002: Balanced translocation disrupted an aminopeptidase gene DACH2	37
DAZL	POI	Deleted in azoospermia-like	RNA-binding protein, essential for gametogenesis in both males and females. Role in spermatogenesis.	3p24	Tung, et al. 2006: Sequenced DAZL in 519 people. Identified 4 putative missense mutations in infertile men and women.	38
DDX17	ANM	DEAD (Asp-Glu-Ala-Asp) box helicase 17	RNA-dependent ATPase, transcriptional regulation, transcriptional coactivator for ESR1.	22q13.1	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs763121, effect -0.16 (0.02), p=2.3E-13	1
DIAPH2	POI	Diaphanous-related formin 2; DIA, DIA2, POF, POF2	Member of diaphanous subfamily of the formin homology family of proteins. May play a role in the development and normal function of the ovaries.	Xq22	Bione, et al. 1998: Gene disrupted by a breakpoint in a family with POF.	39
DIDO1	ANM	Death-inducer obliterator 1	Cytoplasmic protein that translocates to the nucleus on apoptotic signal activation and is upregulated by apoptotic signals. Induces apoptosis.	20q13.33	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs2236553, effect -0.16 (0.03), p=4.4E-10; rs13040088, effect -0.16 (0.02), p=1.9E-11	1
DMC1	ANM	Meiotic recombination protein DMC1/LIM15 homolog	Meiotic recombination, specifically in resolving double-strand breaks.	22q13.1	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs763121, effect -0.16 (0.02), p=2.3E-13	1
	POI	Meiotic recombination protein DMC1/LIM15 homolog	Meiotic recombination, specifically in resolving double-strand breaks.	22q13.1	Mandon-Pepin, et al. 2008: African woman with POF had homozygous missense mutation M200V.	40

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
DMRT1	POI	Doublesex and mab-3 related transcription factor 1 ; CT154, "DM domain expressed in testis 1", DMT1	Transcription factor, testis development and male germ cell proliferation. Represses transcription of female promoting genes, activates male-specific genes.	9p24.3	Bartels, et al. 2013: 31-year-old infertile woman with POI, mild dysmorphic features, a history of mild developmental delay. Deletion of 7.6 Mb from 9p. De novo mutation of paternal origin. DMRT1 is hemizygous.	41
EIF2B2	POI	Eukaryotic translation initiation factor 2B, subunit 2 beta, 39kDa	Subunit of eukaryotic initiation factor 2B (EIF2B), required for GTP exchange during protein synthesis.	14q24.3	Fogli, et al. 2003: Studied 8 patients with POI and white-matter abnormalities in brain. Mutations in EIF2B2: one patient with C512T, 607-12del/insTG (S171F, M203fs); one patient with C547T, A638G (R183stop, E213G).	42
EIF2B3	POI	Eukaryotic translation initiation factor 2B, subunit 3 gamma, 58kDa	Subunit of eukaryotic initiation factor 2B (EIF2B), required for GTP exchange during protein synthesis.	1p34.1	Ghezzi, et al. 2012: A 66-year-old patient with vanishing white matter disease due to the p.Ala87Val EIF2B3 mutation. La Piana, et al. 2012: Sequenced EIF2B3 in woman with POI and leukoencephalopathy. 2 missense mutations: c.260C>T(p.Ala87Val) and c.272G>A(p.Arg91His).	43, 44
EIF2B4	ANM	Eukaryotic translation initiation factor 2B, subunit 4 delta, 67kDa	Subunit of eukaryotic initiation factor 2B (EIF2B), required for GTP exchange during protein synthesis.	2p23.3	He, et al. 2010: Evaluation of 278 candidate genes in 12,723 women (Nurses' Health Study and Women's Genome Health Study studies). P-value adjusted for LD and number of tests. 2 SNPs, smallest p-value os for rs7586601, effect -0.19 years, p=0.0020. Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs704795, effect -0.16 (0.02), p=2.1E-15	12, 1
	POI	Eukaryotic translation initiation factor 2B, subunit 4 delta, 67kDa	Subunit of eukaryotic initiation factor 2B (EIF2B), required for GTP exchange during protein synthesis.	2p23.3	Fogli, et al. 2003: Studied 8 patients with POI and white-matter abnormalities in brain. Mutations in EIF2B4: two patients with C1393T, C1465T (C465R, Y489H).	42
EIF2B5	POI	Eukaryotic translation initiation factor 2B, subunit 5 epsilon, 82kDa	Subunit of eukaryotic initiation factor 2B (EIF2B), required for GTP exchange during protein synthesis.	3q27.1	Fogli, et al. 2003: Studied 8 patients with POI and white-matter abnormalities in brain. Mutations in EIF2B2: two patients with G338A, G338A (R113H, R113H); one patient G338A, C583T (R113H, R195C).	42
EIF3M	ANM	Eukaryotic translation initiation factor 3, subunit M ; eIF3m, FLJ29030, GA17, hfl-B5, TANGO7	Part of the eukaryotic translation initiation factor 3 (eIF-3) complex, required for initiation of protein synthesis.	11p13	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs10734411, effect -0.12 (0.02), p=2.6E-09	1
eIF4ENIF1	POI	Eukaryotic translation initiation factor 4E nuclear import factor 1	Nuclear import of EIF4E, shuttles between nucleus and cytoplasm	22q11.2	Kasippillai, et al. 2013: Family-based genetic study (7 with POI, 1 obligate carrier, 7 unaffected family members), replicate group of women with POI (38 unrelated women). Heterozygous stop codon (Ser429X) in eIF4ENIF1 in affected but not unaffected or unrelated women.	45

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
ESR1	ANM	Oestrogen receptor 1	Oestrogen receptor, ligand activated transcription factor which localises to nucleus and forms a dimer with oestrogen receptor 2.	6q25.1	Weel, et al. 1999: Analysed PvuII restriction enzyme site in 900 women from Rotterdam study. Women homozygous for the PvuII allele (pp) had menopause 1.1 yr later than women homozygous for no PvuII restriction enzyme site rs2234693).	46
	POI	Oestrogen receptor 1	Oestrogen receptor, ligand activated transcription factor which localises to nucleus and forms a dimer with oestrogen receptor 2.	6q25.1	Bretherick, et al. 2008: 55 POF patients, 107 control women from the general population, and 27 control women who had proven fertility after age 37. Repeat in a promoter of the oestrogen receptor alpha(ESR1) gene, POF patients had fewer (<18) short repeat alleles than did controls (P=.004 vs. combined controls). Genotypes consisting of two short alleles were found in 36.4% of control women but only 5.5% of POF patients (P<.0001 vs. combined controls). The ESR1 repeat may confer risk for POF in simple dominant manner in which carriers of long repeat have a relative risk of 9.7 (95% CI = 2.6 - 35.6). Yang, et al. 2010: 100 POF cases and matched 100 EM cases and 200 normal menopause controls from the Korean Multi-Center Cohort. Genotyped XbaI (rs9340799) and PvuII (rs2234693) polymorphisms. Decreased odds of POF for P-x haplotype (OR = 0.5, 95% CI 0.2,0.9) and diplotype (OR = 0.4, 95% CI 0.2,0.9). Cordts, et al. 2012: 70 Brazilian women with POF vs 73 controls, PvuII-rs2234693, p=0.034, OR=2.42 for POF, recessive model. Liu, et al. 2013: 155 Chinese women with idiopathic POF vs 150 healthy controls. Analysis of PvuII (rs2234693) and XbaI (rs9340799) loci using RFLP. P allele of Pvull and X allele of Xbal polymorphisms higher in POF (p=0.008, p=0.011). He, et al. 2015: Meta-analysis of all studies of POF and ESR1 to Aug 2014. 3 studies 487 with POF, 918 controls. Significant association between intron 1 polymorphisms. PvuII-rs2234693: T/C and POF, all genetic models, overall and in Asians. XbaI-rs9340799: A/G significant in Asians, dominant model.	47, 48, 49, 50, 51
EXO1	ANM	Exonuclease 1	5' to 3' double-stranded DNA exonuclease, DNA mismatch repair. Endonuclease activity against 5'-overhanging flap structures.	1q43	Stolk, et al. 2012: HapMap imputed GWAS meta-analysis 38,968 women of European descent, with replication in up to 14,435 women. rs1635501, effect −0.164 years, p=8.46E-10 Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs2236918, effect -0.15 (0.02), p=8.3E-14	18, 1

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
F5	ANM	Coagulation factor V	Critical cofactor for the prothrombinase activity of factor Xa, regulator of homeostasis.	1q23	Tempfer, et al. 2005: Analysed 8 SNPs in 6 genes, 728 white European women. Factor V (F5) Leiden G1691A associated with earlier menopause (p=0.03).	13
FAM175A	ANM	family with sequence similarity 175, member A; BRCA1-A complex subunit Abraxas; ABRA1, FLJ13614	Component of the BRCA1-A complex. Involved in DNA damage response and double-strand break repair.	4q21.23	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs4693089, effect -0.2 (0.02), p=9.2E-23	1
FANCA	POI	Fanconi anaemia complementation group A gene	DNA repair. Part of complex composed of FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL/PHF9 and FANCM missing in Fanconi anaemia patients, who have cytogenetic instability, hypersensitivity to DNA crosslinking agents, increased chromosomal breakage, and defective DNA repair.	16q24.3	Pyun, et al. 2014: 98 Korean women with POF, 218 controls. No single SNPs in FANCA significant at corrected p. 6 SNPs formed a linkage disequilibrium block, three main haplotypes - 2 haplotypes higher frequency in POF group and one higher frequency in controls (highest OR=2.515; 95% CI 1.515-4.175; P = 0.00036).	52
FANCI	ANM	Fanconi anaemia, complementation group I; KIAA1794, FLJ10719	Repair of DNA double-strand breaks by homologous recombination, repair of inter-strand DNA cross-links. Binds branched DNA.	15q26.1	He, et al. 2010: Evaluation of 278 candidate genes in 12,723 women (Nurses' Health Study and Women's Genome Health Study studies). P-value adjusted for LD and number of tests. rs2351002, effect -0.18 years, p=0.019 Stolk, et al. 2012: HapMap imputed GWAS meta-analysis 38,968 women of European descent, with replication in up to 14,435 women. rs2307449, effect −0.184 years, p=3.56E-13. Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs1054875, effect -0.19 (0.02), p=1.7E-19	12; 18, 1
FBXO18	ANM	F-box protein, helicase, 18; FBH1, Fbx18, FLJ14590	ATP-dependent DNA helicase activity. Member of the F-box protein family.	10p15.1	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry, rs10905065, effect -0.11 (0.02), p=3.9E-08	1

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
FIGLA	POI	Folliculogenesis specific basic helix-loop-helix; bHLHc8	Germline specific transcription factor, oocyte-specific gene expression including those initiating folliculogenesis, may also be required for normal ovarian development in adults.	2p13.3	Zhao, et al. 2008: FIGLA oocyte specific gene. Found 3 mutations in 4 females of 100 Chinese females with POF. Missense c.11c-A(p.A4E), deletion c.15-36 del (p.G6fsX66), deletion c.419-421 delACA (p.140delN). Tosh, et al. 2015: 219 Indian women with POF, 230 controls. Analysed FIGLA gene. 7 FIGLA variants detected, including nonsynonymous variant, p.(Arg83Cys) and p.(Ser141Thr).	53, 54
FMR1	POI	FRAXA	Fragile X mental retardation 1 protein, RNA binding protein, expansion of CGG repeat in 5'UTR causes fragile X syndrome.	Xq27.3	Mallolas, et al. 2001: 98 premutation carriers 12.2% POF and 15.3% EM, 6 full carriers with no POF or EM. 43 women with POF - 2 carriers. Gersak, et al. 2003: Screened FMR1 in 83 women with POF, Slovenia. 4/83 had premution (4.8%; 95% CI 1.9,11.7). Allen, et al. 2007: Analysed cross-sectional reproductive history questionnaire data from 948 women. Mid-range premutation repeat size group (80–100 repeats) had an increased risk for: altered cycle traits (shortened cycle length, irregular cycles and skipped cycles), subfertility and dizygotic twinning. Van Esch, et al. 2009: Two sisters compound heterozygous for a premutation with POI at 17 and 22 years. Cronister, et al. 2008: Fragile X DNA analysis for 14,675 women, aged 18 years or older, and 238 mother-offspring pairs between January 1999 and June 2004. 1 in 10 with POI had an FMR1 mutation compared with 1 in 257 for women with no known risk for fragile X. Allen, et al. 2014: 1300 premutation carriers. Increased risk of menopause for repeat of 55-200 copies, even when POF cases excluded: 55-79 copies HR=2.3 (p<0.0001); 80-100 copies HR=3.1 (p<0.0001); 100-200 copies HR=2.2 (p=0.0035). Murray, et al. 2014: Prevalence of the premutation was 2.0% in POI, 0.7% in EM, and 0.4% in controls, OR=5.4 (95% CI 1.7,17.4; P = 0.004) for POI and OR=2.0 (95% CI 0.8, 5.1; P = 0.12) for EM. POI + EM OR=2.4 (95% CI 1.02, 5.8; P = 0.04).	Some recent citations: 55,56, 57, 58, 59, 60

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
FOXE1	POI	Forkhead box protein E1	Thyroid transcription factor, thyroid gland organogenesis.	9q22	Watkins, et al. 2006: Polyalanine tract length in FOXE1 screened in 54 New Zealand POI patients, 71 Slovenian POI patients, 120 New Zealand controls and 37 Slovenian controls. 14 Ala allele less common in POI than controls (p=0.0001), 16 Ala allele more common in POI than controls (p=0.0001). Qin, et al. 2011: 110 Chinese women with idiopathic POF, 110 controls. Screened polyAlanine tract and flanking sequence of FOXE1. 3 lengths of polyAla tract identified - 12, 14 or 16 repeats. 14 Ala allele less common in POI (P = 0.0001), and 16 Ala more common in POI (P = 0.0001). Frequency of homozygotes for 16 higher in POI (p=0.001), homozygotes for 14 lower in POI (p=0.001).	61, 62
FOXL2	POI	Forkhead box protein L2	Transcriptional regulator, critical for ovary differentiation and maintenance, prevents differentiation of ovary to testis.	3q22.3	Harris, et al. 2002: Screened FOXL2 in 70 patients from New Zealand and Slovenia, 100 controls. 30bp deletion in Slovenian patient, (A221_A230del) in poly Ala tract, 772T>A, Y258N in New Zealand patient. Nallathambi, et al. 2007: Polyalanine expansion of +5 residues (FOXL2–Ala19) in an Indian family where heterozygous mutation carriers are unaffected and homozygous individuals have BPES. Correa, et al. 2010: 28 year-old Brazilian woman with hypergonadotropic hypogonadism, c.627delT leading to a truncated protein product. Lin, et al. 2010: Sequenced FOXL2 in Taiwanese patients with BPES. c.855-871dup (17-bp insertion) associated with POF. Fan, et al. 2011: Chinese family with BPES Type 1 including POF, novel missense mutation in the forkhead domain of the FOXL2 gene (c.340A > G, p.K114E). Functional studies confirmed loss-of-function and loss of contacts with StAR. Kim, et al. 2014: Functional studies of FOXL2 mutant cell lines - mutant FOXL2 protein was defective in activating transcription of genes including Caspase8, TNF-R1, FAS, p21 and BMP4, which regulate proliferation, differentiation, and apoptosis of granulosa cells. Martinez-Aguayo, et al. 2014: BPES Type I patient with POF. Heterozygous 11 bp duplication in FOXL2 (c.901_911dup11), predicted to encode a truncated protein (p.Pro305Argfs*54).	63, 64, 65, 66, 67, 68, 69

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
FOXO1A	POI	Forkhead box O1A	Transcription factor, target of insulin signalling, regulates metabolic homeostasisPromotes neural cell death. Regulates adipogenic gene expression.	13q14.1	Watkins, et al. 2006: 30 POF patients from New Zealand, 60 POF patients from Slovenia, 30 controls from New Zealand, 30 controls from Slovenia. Analysis of coding region of FOXO1A and FOXO3A. 6 variants in FOXO1A. Mutations leading to amino acid changes and not found in controls were in 1/90 (1.1%) POF patients.	70
FOXO3	POI	Forkhead box protein O3, FKHRL1, FOXO3A	Transcriptional activator, triggers apoptosis in the absence of survival factors.	6q21	Watkins, et al. 2006: 30 POF patients from New Zealand, 60 POF patients from Slovenia, 30 controls from New Zealand, 30 controls from Slovenia. Analysis of coding region of FOXO1A and FOXO3A. 8 variants in FOXO3A. Mutations leading to amino acid changes and not found in controls were in 2/90 (2.2%) POF patients. Wang, et al. 2010: Screened FOXO3 in 114 Chinese women with POI, 100 controls. Nonsynonymous variants, c.71C>A (p.Pro24His), c.140C>T (p.Pro47Leu), c.184G>A (p.Asp62Asn), c.1652C>T (p.Ser551Phe) and c.1697C>G (p.Gly566Ala), not detected in controls.	70, 71
FSHB	ANM	Follicle-stimulating hormone beta subunit	Pituitary glycoprotein, encodes beta subunit of FSH, stimulates follicle development in ovary.	11p13	He, et al. 2010: Evaluation of 278 candidate genes in 12,723 women (Nurses' Health Study and Women's Genome Health Study studies). P-value adjusted for LD and number of tests. 3 significant SNPs, most significant rs621686, effect 0.32 years, p=0.0071 Stolk, et al. 2012: HapMap imputed GWAS meta-analysis 38,968 women of European descent, with replication in up to 14,435 women. rs12294104, effect 0.225 years, p=1.46E−11. Perry, et al. 2014: Bi-variate analysis of menarche and menopause in 21,505 women, replication in 19,851 women. rs11031002, p=6.16E-11. Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs11031006, effect -0.25 (0.03), p=4.0E-17; rs6484478, effect -0.14 (0.02), p=1.0E-08	12, 18, 72, 1
	POI	Follicle-stimulating hormone beta subunit	Pituitary glycoprotein, encodes beta subunit of FSH, stimulates follicle development in ovary	11p13	Matthews, et al. 1993: Women with homozygous 2 bp frameshift deletion, predicted truncated protein, leads to isolated FSH deficiency causing amenorrhea. Matthews, et al. 1997: Same mutation in an Israeli woman. Layman, et al. 1997: rs5030776, C>G at position 69 leads to isolated FSH deficiency causing amenorrhea. Kumar, et al. 1997: Ovaries and uteri from fshbm1/fshbm1 mice were small and thin. Ovaries lacked corpora lutea and failed to demonstrate any normal follicles beyond the primary (pre-antral) follicle stage - they did not undergo normal oestrous cycles. FSH-deficient females are infertile due to a block in folliculogenesis prior to antral follicle formation.	73, 74, 75, 76

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
FSHR	POI	Follicle stimulating hormone receptor; FSHRO, LGR1	Receptor for FSH, G-protein receptor.	2p21-p16	Aittomaki, et al. 1995: Found a C566T transition in exon 7 of FSHR predicting Ala(189)→ Val in families with hypergonadotropic ovarian dysgenesis. Jiang, et al. 1998: Frequency of an inactivating point mutation (566C-→ T) in FSHR in 4981 women from 4 countries - Finland, Switzerland, Denmark, China. Frequency 0.96% Finnish samples, one Swiss sample, but no others. Touraine, et al. 1999: Woman with normal puberty but primary amenorrhea at age 19. Two heterozygous mutations: Asp224Val in the extracellular domain and Leu601Val in the third extracellular loop of FSHR with functional effects. Doherty, et al. 2002: Novel FSHR mutation 1255G-→ A in a Finnish female with primary amenorrhea, abolished the cAMP second messenger response. Meduri, et al. 2003: Patient with POI, high FSH, very low oestrogen and inhibin B. Novel homozygous Pro(519)Thr mutation in FSHR. Kim, et al. 2011: 98 POF patients, 218 matched controls, investigated associations between SNPs in FSHR and CYP19A1 and POI. OR=5.42 (95% CI 1.96,14.98) for rs4646 (CA+AA) in the 3'UTR of CYP19A1 and missense FSHR SNP rs6166 (AG+GG).OR=5.47 (95% CI 2.03,14.75) for FSHR missense SNP rs6166 (AA) and rs4646-rs10046 CYP19A1 haplotype (C-T)+(C-C). Woad, et al. 2013: Analysed FSHR in 80 New Zealand women with POI and 80 controls. Novel heterozygous FSHR exon 10 variant c.1411A>T p.Ile471Phe in one woman with a family history of POF, but not found in her affected siblings.	77, 78, 79, 80, 81, 33, 82
GAD1	ANM	Glutamate decarboxylase 1	Catalyses production of the neurotransmitter GABA.	2q31.1	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs930036, effect -0.19 (0.02), p=3.1E-19	1
GALT	POI	Galactose-1-phosphate uridylytransferase	Catalyses galactose metabolism Absence of this enzyme results in classic galactosaemia.	9p13	Guerrero, et al. 2000: Analysis of ovarian function in 53 women with classic galactosaemia. Women with galactosaemia more likely to develop POF if patient's genotype is Q188R/Q188R in GALT (p=0.4). Forges, et al. 2006: GALT mutations can cause galactosaemia, hypergonadotrophic hypogonadism in females and galactose-induced ovarian toxicity.	83, 84

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
GDF9	POI	Growth differentiation factor 9	Required for ovarian folliculogenesis. Promotes primordial follicle development. Stimulates granulosa cell proliferation.	5q31.1	Dixit, et al. 2005: Analysis of the GDF9 coding region in 127 Indian women with POI, 58 with primary amenorrhea, 10 with secondary amenorrhea and 220 controls. Two rare missense mutations, c.199A>C (4 POI cases, no controls) and c.646G>A (2 POI cases, 0 controls). Palmer, et al. 2006: GDF9 screened in 279 mothers of DZ twins and 1512 controls. 2 novel insertion/deletions and 4 missense alterations in the GDF9 sequence in mothers of twins. 4.12% of mothers of DZ twins carried any variant - significantly higher (P=0.0001) than controls (2.29%). Laissue, et al. 2006: Sequenced GDF9 and BMP15 in 203 POI patients and 54 controls. Heterozygous missense variant S186Y at conserved position, not found in controls. Kovanci, et al. 2007: Analysis of GDF9 gene in 61 women with POF vs 60 controls. Identified a women with 307C>T 103Pro>Ser in a conserved region of GDF9. Zhao, et al. 2007: Analysed GDF9 coding regions in 100 Chinese women with POI, 96 controls. 4 novel SNPs: Nonsynonymous c.436C>T and c.1283G>C were detected in the control population; c.588A>C (silent); missense c.712A>G (p.Thr238Ala) not present in controls. Norling, et al. 2014: Array CGH analysis of 26 patients with POI, 95 controls, additional 28 POI patients. 11 CNVs, one in promoter region of GDF9.	85, 86, 22, 87, 88,89
GSPT1	ANM	G1 to S phase transition 1 ; Eukaryotic peptide chain release factor GTP-binding subunit ERF3A	Translation termination in response to the codons UAA, UAG and UGA. Involved in regulation of mammalian cell growth.	16p13.13	Stolk, et al. 2012: HapMap imputed GWAS meta-analysis 38,968 women of European descent, with replication in up to 14,435 women. rs10852344, effect 0.168 years, p=1.01E−11. Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs10852344, effect -0.16 (0.02), p=1.3E-15	18, 1
GTF3C2	ANM	General transcription factor IIIC, polypeptide 2, beta 110kDa	Required for RNA polymerase-III mediated transcription. Part of TFIIIC that initiates transcription complex assembly on tRNA.	2p23.3	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs704795, effect -0.16 (0.02), p=2.1E-15	1
HARS2	POI	D-tyrosyl-tRNA(Tyr) deacylase 1; histidyl-tRNA synthetase 2, mitochondrial	ATPase involved in DNA replication.	5q31.3	Pierce, et al. 2011: Non-consanguineous family with 5 affected siblings with Perault syndrome, hearing loss and ovarian dysgenesis. Compound heterozygous for L200V and V368L. L200V results in alternative splice and deletion of 12 codons. Both mutants had reduced aminoacylation.	90

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
HAX1	POI	HCLS1 associated protein X-1; "HCLS1 (and PKD2) associated protein", HCLSBP1, HS1BP1	Promotes cell survival, cell migration, involved in clathrin-mediated endocytosis.	1q21.3	Carlsson, et al. 2013: Two women with severe congenital neutropenia and insufficient pubertal development with p.Glu190X mutation in the HAX1 gene.	91
HDC	ANM	Histidine decarboxylase gene	Catalyses the biosynthesis of histamine from histidine	15q21.2	Zhang, et al. 2006: Studies have suggested that histamine is likely to be involved in the regulation of the reproductive system - stimulates GnRH secretion. Transmission disequilibrium test in 265 postmenopausal Caucasian women from 131 families. Significant within family associations for rs854163 and rs854158 (P values = 0.0018 and 0.0197).	92
HDX	POI	Highly divergent homeobox; chromosome X open reading frame 43, CXorf43	Homeobox protein, specific function unknown.Homeobox genes are involved in embryonic development	Xq21.1	Okten, et al. 2013: 19 year-old with POF, de novo translocation disrupting HDX.	93
HELB	ANM	DNA helicase B	Unwinds duplex DNA with 5' to 3' polarity. Has single-strand DNA-dependent ATPase and DNA helicase activities.	12q14.3	Day, et al. 2015: Exome chip GWAS, 39,026 women, European ancestry: rs75770066, effect=0.85 (0.07), p=1.17E-31; rs148126992, effect=1.04 (0.09), p=1.69E-30; HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs1183272, effect -0.31 (0.03), p=3.0E-24; rs1183272, effect -0.31 (0.03), p=3.0E-24; rs7397861, effect -0.13 (0.02), p=4.6E-09	1
HELQ	ANM	Helicase, POLQ-like	Single-stranded DNA-dependent ATPase and DNA helicase.	4q21.23	Stolk, et al. 2012: HapMap imputed GWAS meta-analysis 38,968 women of European descent, with replication in up to 14,435 women. rs4693089, effect 0.228, p=2.38E−19 Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs4693089, effect -0.2 (0.02), p=9.2E-23	18, 1
HFM1	POI	HFM1, ATP-dependent DNA helicase homolog (S. cerevisiae)	Homologous recombination during meiosis, required for cross-over formation and complete synapsis.	1p22.2	Wang, et al. 2014: 2 sisters with compound heterozygous mutation in HFM1. Sequencing HFM1 in 69 Chinese women with sporadic POI identified another heterozygous mutation not found in 216 controls. HFM1 required for homologous recombination in mice. All four mutations identified are predicted to have functional effects.	94

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
HLA genes	ANM	Human leukocyte antigen genes	Antigen presentation, immune function	6p21.33	Stolk, et al. 2012: HapMap imputed GWAS meta-analysis 38,968 women of European descent, with replication in up to 14,435 women. rs1046089, effect −0.213 years, p=1.63E−16. Perry, et al. 2014: Bi-variate analysis of menarche and menopause in 21,505 women, replication in 19,851 women. rs2471980, p=2.59E-14. Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs2230365, effect -0.16 (0.03), p=2.7E-08; rs707938, effect -0.16 (0.02), p=2.3E-13	18, 72, 1,
	POI	Human leukocyte antigen genes	Antigen presentation, immune function	6p21.33	Arif, et al. 1999: Analysed HLA-DRB1 and -DQB1 genotypes in 118 women with POF and 134 racially matched control subjects. Two HLA-DQB1 alleles, 0301 and 0603 were associated with 3beta-HSD autoantibody positivity (P=0.04; P=0.006). Ruggeri, et al. 2013: Patients with Grave's disease and POI found to have HLA previously described for POF HLA-DRB1*11/ DQB1*0301.	95, 96
HSD17B4	POI	Hydroxysteroid (17-beta) dehydrogenase 4; Peroxisomal multifunctional enzyme type 2	Part of the beta-oxidation pathway for fatty acids.	5q2	Pierce, et al. 2010: Family of mixed European ancestry, two sisters with Perault syndrome including ovarian dysgenesis. Sisters are compound heterozygotes for HSD17B4 c.650A>G (p.Y217C) (maternal allele), predicted to destabilise protein, and HSB17B4 c.1704T>A (p.Y568X) (paternal allele), predicted to have low levels of transcript.	97
IGF1	ANM	Insulin-like growth factor I	Growth factor stimulating cellular proliferation and differentiation	12q23.2	He, et al. 2010: Evaluation of 278 candidate genes in 12,723 women (Nurses' Health Study and Women's Genome Health Study studies). P-value adjusted for LD and number of tests. rs1019731, effect -0.28 years, p=0.0053	12
IGF2R	ANM	Insulin-like growth factor 2 receptor; CD222, CIMPR, M6P-R, MPR1, MPRI, Cation-independent mannose-6-phosphate receptor	Binds IGF2, binds phosphorylated lysosomal enzymes in the Golgi apparatus facilitating transport to lysosomes.	6q25.3	He, et al. 2010: Evaluation of 278 candidate genes in 12,723 women (Nurses' Health Study and Women's Genome Health Study studies). P-value adjusted for LD and number of tests. 2 SNPs, smallest p-value rs1019731, effect -0.28 years, p=0.0053. Pyun, et al. 2013: Analysed interactions between SNPs and diplotypes in IGF2R and ADAMTS19. 120 Korean patients with POF and 152 controls, plus an additional 1641 female controls. 4 combinations of SNPs in IGF2R and ADAMTS19 were significant (p<0.0071).	12, 4

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
INHA	POI	Inhibin alpha chain	Inhibits secretion of FSH by the pituitary. Part of inhibin A and inhibin B.	2q35	Shelling, et al. 2000: Screened INHA, INHBA, INHBB in 43 women with POF. Found 769G→ A in INHA in 3 patients, non-conservative aa change from Ala to Thr. Variant is in 3/43 (7%) POI patients vs 1/150 (0.7% controls) (p<0.035). Marozzi, et al. 2002: Analysed 157 patients with POI for 769G→ A missense mutation in INHA. Mutation was more frequent in POI (p=0.03). Higher prevalence of the C allele of SNP (129C-→ T), the 5'-UTR of the INHA in POI patients (80.3%) than controls (66.7%) (Fisher's exact test, P= 0.014). Dixit, et al. 2004: Screened INHA in 80 patients with POF, 33 with primary amenorrhoea, 4 secondary amenorrhoea, 100 controls. Ala257Thr missense mutation in POF (9/80, P=0.0005), primary amenorrhoea (3/33, P=0.014) and secondary amenorrhoea (2/4, P=0.001), not in controls. Harris, et al. 2005: Screened for INHA -16C>T transition mutation in 70 POF patients and 70 controls. T allele lower in POF patients (P= 0.033) - 31/70 (44.3%) controls vs 18/70 (25.7%) POF. Sequence analysis of the INHA promoter in 50 POF patients and 50 controls identified a highly polymorphic imperfect TG repeat at ~ -300 bp. 4 common haplotypes (A, B, C and D). The -16T allele is linked to the shortest repeat haplotype (haplotype C) but no significant difference in promoter activity. Prakash, et al. 2009: 100 Indian women with POI, 50 controls. Analysed INHA gene. 769G→ A missense inhibin alpha mutation. Three inhibin alpha gene sequence variants 734 C→ A/Ala 245 Asp, 755 C→ A/Pro 252 His, and 777 C→ A/His 259 Gln. Kim, et al. 2011: 52 Korean women with POF vs 55 healthy controls. INHα -16C/T and -124A/G polymorphisms associated with POF.	98, 99, 100, 101, 102, 103
INHBA	POI	Inhibin beta A chain	Inhibits secretion of FSH by the pituitary. Part of inhibin A.	7p15-p13	Shelling, et al. 2000: Screened INHA, INHBA, INHBB in 43 women with POF. Found 1032C→ T in INHBA in 1 patients but no effect on aa sequence of protein.	98
INO80	ANM	INO80 complex subunit	DNA helicase, component of the chromatin remodelling INO80 complex which is involved in transcriptional regulation, DNA replication and probably DNA repair.	15q15.1	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs9796, effect -0.13 (0.02), p=1.3E-10	1

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
KISS1R	ANM	KISS1 receptor	Receptor for metastin, involved in suppression of metastasis. Regulation of the gonadotropic axis at puberty and in adulthood.	19p13.3	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs349306, effect -0.23 (0.04), p=1.7E-10	1
KNTC1	ANM	Kinetochore-associated protein 1	Stops cells from prematurely exiting mitosis, required for assembly of dynein-dynactin and MAD1-MAD2 complexes onto the kinetochores.	12q24.31	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs1727326, effect -0.19 (0.03), p=1.7E-09	1
LAMC1	POI	Laminin, gamma 1 (formerly LAMB2)	Extracellular matrix glycoprotein. Role in cell adhesion, differentiation, migration, signalling.	1q31	Pyun, et al. 2012: GWAS study on 24 cases and 24 controls, 2 haplotypes 1q31 associated with POF. Genotyped 14 SNPs in 98 patients and 218 controls, distributions of 9 SNPs higher in cases than controls (86.6% vs 74.5%) OR= 2.209 (95% CI 1.139,4.284, P = 0.017).	104
LARS2	POI	Probable leucine--tRNA ligase, mitochondrial; leucyl-tRNA synthetase 2, mitochondrial	Catalyses the aminoacylation of a specific tRNA	3p21.3	Pierce, et al. 2013: Two families with POF and hearing loss (Perrault syndrome), homozygous c.1565C>A (p.Thr522Asn), in a consanguineous Palestinian family; compound heterozygous c.1077delT and c.1886C>T (p.Thr629Met) in a nonconsanguineous Slovenian family.	105
LEP	POI	Leptin, OB, OBS	Part of signalling pathway that regulates body fat stores.	7q31	Kim, et al. 2012: 852 women (400 from breast cancer study, 452 healthy) in Korea. Investigated associations between 10 SNPs and in LEP, LEPR, and PPARγ and age at menopause. rs2167270 of LEP and rs1801282, rs2120825, and rs3856806 of PPARγ were associated with menopause under 40.	106
LHB	POI	Luteinizing hormone subunit beta	Reproductive hormone, stimulates testes and ovary to synthesise steroids.	19q13	Takahashi, et al. 2001: Woman with severe ovarian dysfunction and infertility, heterozygosity for point mutations Trp(8) to Arg(8) and Ile(15) to Thr(15).	107
LHCGR	ANM	Luteinizing hormone/choriogonadotropin receptor	Receptor for luteinizing hormone/choriogonadotropin .	2p21	He, et al. 2010: Evaluation of 278 candidate genes in 12,723 women (Nurses' Health Study and Women's Genome Health Study studies). P-value adjusted for LD and number of tests. rs1464729, effect 0.23 years, p=0.0036	12
	POI	Luteinizing hormone/choriogonadotropin receptor; LCGR, LGR2, LHR, ULG5, HHG	Receptor for LH. G-protein coupled receptor.	2p21	Latronico, et al. 1996: Family 1: 3 pseudohermaphrodite 46,XY siblings with Leydig-cell hypoplasia, a 46,XX sister with amenorrhea (small uterus and cystic ovaries of unequal sizes). Family 2: boy with micropenis and primary hypogonadism. Two novel homozygous inactivating nonsense and missense mutations of the LH-receptor gene - Arg554 stop codon554 (TGA) and Ser616 Tyr616.	108

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
LMNA	POI	Lamin A/C ; HGPS; "cardiomyopathy, dilated 1A (autosomal dominant)", CMD1A, "lamin A/C-like 1", LGMD1B, "limb girdle muscular dystrophy 1B (autosomal dominant)", LMN1, LMNL1, PRO1, "progeria 1 (Hutchinson-Gilford type)"	Part of the nuclear lamina, a fibrous layer on the nucleoplasmic side of the inner nuclear membrane. Role in nuclear assembly, chromatin organization, nuclear membrane and telomere dynamics.	1q22	McPherson, et al. 2009: 2 unrelated women with dysmorphic features, cardiomyopathy, and POI. Both had heterozygous mutation c.176T>G in LMNA gene, resulting in Leu59Arg.	109
MAK	ANM	Male germ cell-associated kinase; dJ417M14.2, RP62	Phosphorylates FZR1 in a cell cycle-dependent manner, role in the transcriptional co-activation of AR, suggested function in spermatogenesis.	6p24.2	He, et al. 2009: GWAS in 9,112 Europeans (Nurses' Health Study, Women's Genome Health Study studies). Rs2153157, effect 0.29, p=5.1E-08 Stolk, et al. 2012: HapMap imputed GWAS meta-analysis 38,968 women of European descent, with replication in up to 14,435 women. rs2153157, effect 0.165 years, p=7.76E−12. Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs6899676, effect -0.21 (0.03), p=6.2E-16; rs9393800, effect -0.14 (0.02), p=1.1E-09	25, 18, 1
MCM8	ANM	Minichromosome maintenance complex component 8	DNA helicase. Homologous recombination.	20p12.3	He, et al. 2009: GWAS in 9,112 Europeans (Nurses' Health Study, Women's Genome Health Study studies). rs16991615, effect 1.07 years, p=1.2E-21 Stolk, et al. 2009: GWAS in 2,979 Europeans (Rotterdam, Twins UK studies), rs236114, effect 0.5, p=9.71E–11 Chen, et al. 2012: Replication GWAS in 3468 Hispanic women, rs16991615, effect -2.3 years, p=1.4E-06 Stolk, et al. 2012: HapMap imputed GWAS meta-analysis 38,968 women of European descent, with replication in up to 14,435 women. rs16991615, effect 0.948 years, p=1.42E−73. Chen, et al. 2014: Replication GWAS in 6510 African American women, rs6139882, effect -0.85 years, p=1.35E-03 Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs451417, effect -0.2 (0.03), p=4.5E-09; rs16991615, effect -0.88 (0.04), p=4.4E-89	25, 17, 26, 18, 11, 1

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
	POI	Minichromosome maintenance complex component 8	DNA helicase. Homologous recombination.	20p12.3	AlAsiri, et al. 2015: SNP analysis, whole exome sequencing of 3 sisters with primary amenorrhea, hypothyroidism, and hypergonadotropic hypogonadism from consanguineous family. Autosomal recessive mutation: MCM8, c.446C>G; p.P149R in homozygous region. Double strand break repair deficient in fibroblasts from affected sisters compared with unaffected family members.	110
MCM9	POI	Minichromosome maintenance complex component 9	DNA helicase, homologous recombination.	6q22.31	Wood-Trageser, et al. 2014: Two unrelated consanguineous families with daughters with primary amenorrhea, short stature, who were 46,XX. Two variants identified: MCM9 c.1732+2T>C alters a splice donor site, resulting in truncated MCM9 protein that is unable to be recruited to sites of DNA damage; MCM9 c.394C>T (p.Arg132*) results in a predicted loss of functional MCM9. In affected females from both families, repair of chromosome breaks impaired in lymphocytes.	111
MSH5	ANM	mutS homolog 5	Involved in meiotic recombination, needed for crossovers between homologs.	6p21.33	Stolk, et al. 2012: HapMap imputed GWAS meta-analysis 38,968 women of European descent, with replication in up to 14,435 women. rs1046089, effect −0.213 years, p=1.63E−16. Perry, et al. 2014: Bi-variate analysis of menarche and menopause in 21,505 women, replication in 19,851 women. rs2471980, p=2.59E-14. Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs2230365, effect -0.16 (0.03), p=2.7E-08; rs707938, effect -0.16 (0.02), p=2.3E-13	18, 72, 1
	POI	mutS homolog 5	Involved in meiotic recombination, needed for crossovers between homologs.	6p21.3	Mandon-Pepin, et al. 2008: 41 women with POF, 36 controls. Heterozygous miss-sese mutation, P29S in MSH55, found in 2 Caucasian women with POF.	40
MSH6	ANM	DNA mismatch repair protein Msh6	DNA mismatch repair, forms heterodimer with MSH2.	2p16.3	Perry, et al. 2014: Bi-variate analysis of menarche and menopause in 21,505 women, replication in 19,851 women, rs1800932, effect=1.3 months, p=1.9E-09. Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs1800932, effect -0.17 (0.03), p=3.2E-11	72, 1
MT-ATP6	POI	ATP synthase subunit a	Mitochondrial membrane ATP synthase, produces ATP from ADP from a proton gradient across the mitochondrial membrane which is generated by electron-transport during respiration.	mt	Venkatesh, et al. 2010: 20 cases of POI, 20 controls. Sequenced mt ATPase6. 50% POI patients has mutations in ATPase6 vs 10% controls. Non-synonymous ATPase6 mt.8684 C>T and mt.9094 C>T higher in cases than controls (p<0.005). Reactive oxygen species higher in POI vs controls (p<0.005).	112

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
MTHFR	ANM	Methylenetetrahydrofolate reductase	catalyses the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate.	1p36.3	Liu, et al. 2010: Study of association of 6 SNPs in MTHFR with ANM, rs2066470, rs17037390, rs1801133, rs1476413, rs4846048, rs4846049. 4 haplotypes associated with ANM ($p<0.04$), all include rs1476413.	113
MYCBP	ANM	MYC binding protein; c-myc binding protein; AMY-1, associate of myc-1	E3 ubiquitin-protein ligase.Possible role during synaptogenesis.	1p34.3	Stolk, et al. 2012: GWAS meta-analysis 38,968 women of European descent, with replication in up to 14,435 women. rs4246511, effect 0.24 years, $p=9.08E{-}17$ Chen, et al. 2014: Replication GWAS in 6510 African American women, rs4246511, effect 0.21 years, $p=0.031$ Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs4246511, effect -0.22 (0.02), p=5.1E-21	18, 11, 1
NANOS3	POI	nanos homolog 3 (Drosophila); NANOS1L, NOS3	Possible translational repressor. Regulates translation. Required for primordial germ cell development and maintenance in other organisms.	19p13.12	Qin, et al. 2007: Sequenced 80 Chinese and 88 Caucasian women with POI. Only a known synonymous SNP (rs2016163) was identified. Wu, et al. 2013: Screened coding regions of NANOS1, NANOS2, NANOS3 in 100 Chinese POI patients. Identified one mutation in NANOS3 (c.457C4T; p.Arg153Trp,heterozygous), decreases the stability of NANOS3. Santos, et al. 2014: Analysis of NANOS3 in 85 Brazilian women with POI and matched controls. Homozygous p.Glu120Lys mutation in NANOS3 in two sisters with primary amenorrhea, impairs ability of NANOS3 to prevent apoptosis.	114, 115, 116
NBN	ANM	Nibrin; NBS, NBS1, "Nijmegen breakage syndrome 1 (nibrin)" ;	DNA damage response, member of the MRE11/RAD50 double-strand break repair complex, mutations are associated with Nijmegen breakage syndrome.	8q21-q24	He, et al. 2010: Evaluation of 278 candidate genes in 12,723 women (Nurses' Health Study and Women's Genome Health Study studies). P-value adjusted for LD and number of tests. 3 SNPs, smallest p rs2697679, effect -0.24 years, $p=0.00021$.	12
	POI	Nibrin; NBS, NBS1, "Nijmegen breakage syndrome 1 (nibrin)" ;	DNA damage response, member of the MRE11/RAD50 double-strand break repair complex, mutations are associated with Nijmegen breakage syndrome.	8q21-q24	Chrzanowska, et al. 2010: 37 girls and young women with Nijmegen breakage syndrome (NBS) homozygous for c.657_661del5 mutation had POI / hypergonadotropic hypergonadism.	117

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
NLRP11	ANM	NACHT, LRR and PYD domains-containing protein 11	Involved in inflammation.	19q13.42	He, et al. 2009: GWAS in 9,112 Europeans (Nurses' Health Study, Women's Genome Health Study studies). 6 SNPs at this locus. rs1172822 had smallest p, effect -0.49 years, p=1.8E-19. Candidates: BRSK1, HSPBP1, SUV420H2. Stolk, et al. 2009: GWAS in 2,979 Europeans (Rotterdam, Twins UK studies). 4 SNPs at this locus. rs1172822 had smallest p, effect=-0.4 years, p=6.28E-11. Candidates: BRSK1, TMEM224, SUV420H2. Stolk, et al. 2012: GWAS meta-analysis in 38,968 Europeans, replication in 14,435. rs11668344, effect -0.4 years, p=1.45E10-59. Candidate: TMEM150B. Other genes: BRSK1, HSPBP1, COX6B2, LOC284417, IL11, SUV420H2 Chen, et al. 2012: Replication GWAS in 3468 Hispanic women, rs17782355, effect -1.4 years, p=0.0064. Shen, et al. 2013: Replicated rs7246479 in GWAS of 3,533 Chinese women. Effect= 0.49 years, p=3.75E-05. Replicated rs1172822. Effect= -0.6 years, p=6.64E-05. Chen, et al. 2014: Replication GWAS in 6510 African American women, rs11668344, effect -0.31 years, p=6.54E-04 Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs11668344, effect -0.41 (0.02), p=4.2E-84; rs2547274, effect -0.22 (0.04), p=2.7E-08; rs12461110, effect -0.15 (0.02), p=5.0E-14	1; 25, 17, 26, 27, 11
NOBOX	POI	NOBOX oogenesis homeobox	Transcription factor, essential for folliculogenesis and regulation of oocyte-specific genes in mice	7q35	Qin, et al. 2007: Sequenced NOBOX in 96 white women with POF. 7 SNPs (rs757388, rs11769847, rs11979528), 4 novel mutations of which 2 (p.Arg355His, p.Arg360Gln) cause missense mutations. p.Arg355His disrupts NOBOX binding to DNA. Bouilly, et al. 2011: 178 women with idiopathic POI; 362 ethnically matched controls. Sequenced exons of NOBOX, functional studies on mutants (protein expression, reporter assays). 19 variants in 12 Caucasian and African patients. 5 variants not seen before, all heterozygous: 1 nonsense (c.907C>T/p.R303X); 4 missense (c.271G>T/p.G91W, c.349C>T/p.R117W, c.1025G>C/p.S342T, and c.1048G>T/p.V350L). All have functional effects. Absence of NOBOX causes sterility in mice.	118, 119
NOG	POI	Noggin	Inhibits bone morphogenic protein (BMP) signalling.	17q22	Kosaki, et al. 2004: Japanese POF patient diagnosed with POF found to have heterozygous mutation (E48K) in Noggin. Kadi, et al. 2012: Women with primary amenorrhea and proximal symphalangism (fusion of joints in fingers). NOG mutation has been identified as causing proximal symphalangism.	120, 121

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
NR5A1	POI	Nuclear receptor subfamily 5, group a, member 1 / Steroidogenic factor 1, AD4BP, ELP, FTZ1, SF-1	Transcriptional regulator of genes involved in hypothalamic-pituitary-gonadal axis and adrenal axis	9q33	Lourenco, et al. 2009: Sequenced NR5A1 in four families with histories of both 46,XY disorders of sex development and 46,XX POI, and 25 subjects with sporadic ovarian insufficiency, 700 controls. In-frame deletions, frameshift and missense mutations in NR5A1 in members of all 4 families and 2/25 subjects with isolated ovarian insufficiency, which impaired transactivational activity of NR5A1. Ciaccio, et al. 2012: Studied family with males and females with gonadal dysgenesis, 3 with POI, heterozygous mutation c.938G→ A, predicted to cause a p.Arg313Hysamino acid change. Lakhal, et al. 2012: Sequenced the coding region of WNT4 and SF1 in 55 Tunisian women with POF and 100 healthy controls. SF1 Gly146Ala polymorphism significantly higher (p=0.029) in POI patients versus controls, associated with reduction in plasma oestradiol levels. Janse, et al. 2012: Sequenced coding region and splice sites of NR5A1 in 386 women with secondary amenorrhea (of which 77 with familial POI), 5 novel non-conservative mutations in 5 patients. Camats, et al. 2012: Two women with POI from Switzerland. Heterozygous mutation in SF-1/NR5A1 c.704C>T; Pro235Leu. Jiao, et al. 2013: Mutation screen of NR5A1 in 400 Han Chinese women with idiopathic non-syndromic POF and 400 controls. Identified heterozygous novel mutation in 1 patient: c.13T.G (p.Tyr5Asp) in NR5A1 which impaired functional activity. Harrison, et al. 2013: 0.23 Mb microdeletion in 9q33.3 including NR5A1 causing POF in mother and disorder of sex development in son. Philibert, et al. 2013: 26 cases of POI, analysis of NR5A1, novel mutation c.763C>T (p.Arg255Cys) with functional effects, known variant c.437G>C (p.Gly146Ala) of higher frequency in cases (46%) than controls (10%). Fabbri, et al. 2014: Brazilian family with a novel NR5A1 mutation ambiguous genitalia in 46,XY affected individuals, mother with POI. Heterozygous for p.Cys65Tyr mutation Eggers, et al. 2015: Large family with 46,XY DSD and 46,XX POI, exome capture and massively parallel sequencing, novel, 3 bp, in-frame deletion in exon six of NR5A1 at lys 372, results in truncated protein that fails to bind and activate downstream targets of SF-1.	122, 123, 124, 125,126, 127, 128, 129, 130, 131

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
NXF5	POI	Nuclear RNA export factor 5	RNA binding protein implicated in mRNA nuclear export.	Xq22.1	Bertini, et al. 2010: To characterize the breakpoints of a t(X;15) found in a 19 year-old woman with primary amenorrhea. Chromosome and FISH analysis revealed 46,XX, t(X;15)(Xq22.1;p11). FISH showed that NXF5 (nuclear RNA export factor 5) gene was contained in the clone spanning the breakpoint on the X chromosome.	132
PAI-1	POI	Plasminogen activator inhibitor-1, SERPINE1, PLANH1	Serine protease inhibitor, suggested involvement in control of fibrinolysis.	7q22.1	Jeon, et al. 2014: Genotyping of 5 PAI-1 polymorphisms rs2227631, rs1799889, rs6092, rs2227694, rs7242 in 137 POI cases and 227 controls. SNPs were associated with POI. Mechanism unknown.	133
PAPD7	ANM	Non-canonical poly(A) RNA polymerase PAPD7	Post-transcriptional quality control, poly(A) polymerase activity.	5p15.31	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs427394, effect -0.13 (0.02), p=3.8E-09	1
PARL	ANM	Presenilins-associated rhomboid-like protein, mitochondrial	Control of apoptosis during post-natal growth.	3q27.1	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs16858210, effect -0.14 (0.02), p=3.1E-09	1
PARP2	ANM	Poly [ADP-ribose] polymerase 2	Involved in base excision repair pathway, catalyses poly(ADP-ribosyl)ation of acceptor proteins involved in chromatin architecture and in DNA metabolism following DNA damage.	14q11.2	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs1713460, effect -0.14 (0.02), p=2.4E-10	1
PCSK1	ANM	Proprotein convertase subtilisin/kexin type 1; "neuroendocrine convertase 1", PC1, PC3, "prohormone convertase 1", "prohormone convertase 3", "proprotein convertase 1", SPC3	Processes hormones at pairs of basic amino acids.	5q15-q21	He, et al. 2010: Evaluation of 278 candidate genes in 12,723 women (Nurses' Health Study and Women's Genome Health Study studies). P-value adjusted for LD and number of tests. rs271924, effect -0.18 years, p=0.034	12
PGAP3	ANM	Post-GPI attachment to proteins factor 3	Involved in GPI anchor formation.	17q12	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs2941505, effect -0.13 (0.02), p=1.9E-09	1
PGR	ANM	Progesterone receptor, NR3C3, PR	Progesterone receptor, involved in cellular differentiation and proliferation.	11q22-q23	He, et al. 2010: Evaluation of 278 candidate genes in 12,723 women (Nurses' Health Study and Women's Genome Health Study studies). P-value adjusted for LD and number of tests. rs619487, effect 0.19 years, p=0.026	12

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
PGRMC1	POI	Progesterone receptor membrane component-1	Mediates anti-apoptotic action of progesterone in ovarian cells, regulator of steroid hormone biosynthesis.	Xq22-q24	Mansouri, et al. 2008: X-autosome translocation [t(X;11)(q24;q13)] identified in mother & daughter with POF. H165R mutation in female with idiopathic POF. Reduced levels of PGMRC1 may cause POF through impaired activation of P450 and increased apoptosis of ovarian cells.	134
PITPNM2	ANM	Membrane-associated phosphatidylinositol transfer protein 2	Catalyses the transfer of phosphatidylinositol and phosphatidylcholine between membranes.	12q24.31	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs1727326, effect -0.19 (0.03), p=1.7E-09	1
PIWIL1	ANM	Piwi-like protein 1	Central role during spermatogenesis by repressing transposable elements.	12q24.33	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs12824058, effect -0.14 (0.02), p=6.1E-11	1
POF1B	POI	Protein POF1B	Regulates actin cytoskeleton, organisation of epithelial monolayers, possible role in ovary development.	Xq21	Lacombe, et al. 2006: Lebanese family with 5 sisters with POF, found to be homozygous mutation (R329Q) in exon 10. Mutation alters POF1B binding to non-muscle actin filaments suggesting a function in germ-cell division.	135
POLG	ANM	DNA polymerase subunit gamma-1; POLG1, POLGA	Replication of mitochondrial DNA	15q24	He, et al. 2010: Evaluation of 278 candidate genes in 12,723 women (Nurses' Health Study and Women's Genome Health Study studies). P-value adjusted for LD and number of tests. rs2351002, effect -0.18 years, p=0.019 Stolk, et al. 2012: HapMap imputed GWAS meta-analysis 38,968 women of European descent, with replication in up to 14,435 women. rs2307449, effect −0.184 years, p=3.56E-13. Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs1054875, effect -0.19 (0.02), p=1.7E-19	12; 18, 1
	POI	DNA polymerase subunit gamma-1; POLG1, POLGA	Replication of mitochondrial DNA	15q24	Luoma, et al. 2004: Mutations in POLG found in 7 families, women with progressive external ophthalmoplegia had menopause before 35yrs. Pagnamenta, et al. 2006: Patient, mother and maternal grandmother with POI and progressive external ophthalmoplegia. Dominant Y955C mutation. mtDNA depletion in fibroblasts. Reduced DNA polymerase gamma activity in fibroblasts. Blok, et al. 2009: Dominant chronic progressive external ophthalmoplegia (CPEO) mutation (p.R943H) in POLG found to be associated with POF. Dutch patient cohort of 232 patients	136, 137, 138

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
POLR2E	ANM	DNA-directed RNA polymerases I, II, and III subunit RPABC1	Component of DNA-dependent RNA polymerase. Part of lower jaw of RNA polymerase that attaches to incoming DNA template.	19p13.3	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs349306, effect -0.23 (0.04), p=1.7E-10	1
POLR2H	ANM	DNA-directed RNA polymerases I, II, and III subunit RPABC3	Component of DNA-dependent RNA polymerase.	3q27.1	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs16858210, effect -0.14 (0.02), p=3.1E-09	1
POU5F1	POI	POU domain, class 5, transcription factor 1; OCT4	Transcription factors, controls genes involved in embryonic development.	6p21.31	Wang, et al. 2011: Exon sequencing of POU5F1 in 115 POF cases and 149 controls. Non-synonymous variant of POU5F1 (c. C37A, p. Pro13Thr) identified.	139
PPARG	ANM	Peroxisome proliferator-activated receptor gamma; NR1C3, PPARG1, PPARG2, PPARgamma	Activated by ligand to bind to DNA response elements, involved in adipocyte differentiation and glucose homeostasis, controls the peroxisomal beta-oxidation pathway of fatty acids.	3p25	He, et al. 2010: Evaluation of 278 candidate genes in 12,723 women (Nurses' Health Study and Women's Genome Health Study studies). P-value adjusted for LD and number of tests. rs4135280, effect 0.54 years, p=0.0048.	12
PPARG	POI	Peroxisome proliferator-activated receptor gamma; NR1C3, PPARG1, PPARG2, PPARgamma	Activated by ligand to bind to DNA response elements, involved in adipocyte differentiation and glucose homeostasis, controls the peroxisomal beta-oxidation pathway of fatty acids.	3p25	Kim, et al. 2012: 852 women (400 from breast cancer study, 452 healthy) in Korea. Investigated associations between 10 SNPs and in LEP, LEPR, and PPARγ and age at menopause. rs2167270 of LEP and rs1801282, rs2120825, and rs3856806 of PPARγ were associated with menopause under 40.	106
PPY	ANM	PNP, Pancreatic prohormone	Regulates pancreatic and gastrointestinal functions, synthesised in pancreas.	14q11.2	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs1713460, effect -0.14 (0.02), p=2.4E-10	1
PRIM1	ANM	DNA primase small subunit	Synthesises small RNA primers for Okazaki fragment synthesis during lagging strand DNA replication.	12q13.3	Stolk, et al. 2012: HapMap imputed GWAS meta-analysis 38,968 women of European descent, with replication in up to 14,435 women. rs2277339, effect −0.380 years, p=2.47E-19. Chen, et al. 2014: Replication GWAS in 6510 African American women, rs2277339, effect 0.36 years, p=0.022 Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs2277339, effect -0.31 (0.03), p=1.8E-19	18, 11, 1

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
PSMC3IP	POI	PSMC3 interacting protein; GT198, HUMGT198A, TBP-1 interacting protein, TBPIP	Role in meiotic recombination, stimulates strand exchange .	17q21.2	Zangen, et al. 2011: Homozygosity mapping, and candidate-gene and whole-exome sequencing in a consanguineous Palestinian family with XX female gonadal dysgenesis. Affected females homozygous for a 3 bp deletion (NM_016556.2, c.600_602del) in PSMC3IP gene (p.Glu201del) in the highly conserved C-terminal acidic domain which abolished PSMC3IP activation of oestrogen-driven transcription in cell lines. Candidate gene for POI.	140
PTHB1	POI	Parathyroid hormone responsive B1 gene, BBS9, Bardet-Biedl syndrome 9	Thought to be involved in parathyroid hormone action in bones.	7p14	Kang, et al. 2008: LD-based genome-wide association study 24 pairs of patients with POF and 24 matched controls, BeadChip assay with 109365 SNPs identified 7p14 containing PTHB1 as associated with POF. Region was tested in 101 cases and 87 controls: identified POF-susceptible haplotype (ht1, 'GAAAG', P = 0.00034), POF-resistant haplotype (ht2, 'TGTGC'), association of POF with rs3884597 and rs6944723. rs11773504 Ala>Thr considered as a putative causal variant.	141
RAD51	ANM	DNA repair protein RAD51 homolog 1	DNA damage response, activation of homologous recombination and double strand break repair.	15q15.1	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs9796, effect -0.13 (0.02), p=1.3E-10	1
RAD54L	ANM	DNA repair and recombination protein RAD54-like	DNA repair, mitotic recombination (RAD52 pathway), possible role in telomere maintenance.	1p33	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs12142240, effect -0.13 (0.02), p=6.6E-09	1
RET	POI	Proto-oncogene tyrosine-protein kinase receptor Ret	Receptor tyrosine-protein kinase, involved in cell growth, proliferation, differentiation.	10q11	Orgiana, et al. 2004: RET point mutation [R694Q (CGG→ CAG)] in woman with hypothyroidism due to atrophic Hashimoto's thyroiditis and POI.	142
REV3L	ANM	DNA polymerase zeta catalytic subunit	Part of DNA polymerase zeta, involved in trans-lesion DNA synthesis.	6q21	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs12196873, effect -0.16 (0.03), p=2.8E-08	1
RHBDL2	ANM	Homo sapiens rhomboid, veinlet-like 2 (Drosophila)	Releases functional polypeptides from their membrane anchors, intramembrane proteolysis.	1p34.3	Stolk, et al. 2012: GWAS meta-analysis 38,968 women of European descent, with replication in up to 14,435 women. rs4246511, effect 0.24 years, p=9.08E−17 Shen, et al. 2013: Replicated rs4246511 (RHBDL2) in GWAS of 3,533 Chinese women. Effect= 0.29 years, p=0.002. Chen, et al. 2014: Replication GWAS in 6510 African American women, rs4246511, effect 0.21 years, p=0.031 Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs4246511, effect -0.22 (0.02), p=5.1E-21	18, 27, 11, 1

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
RPAIN	ANM	RPA-interacting protein	Mediates import of RPA protein into the nucleus, which is required to stabilise single strand DNA formed during DNA replication or damage.	17p13.2	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs8070740, effect -0.15 (0.02), p=1.5E-09	1
RPL10	POI	60S ribosomal protein L10, QM	Subunit of ribosome responsible for protein synthesis.	Xq28	Massad-Costa, et al. 2007: Analysis of QM gene in 23 Brazilian women with POI, 14 with gonadal dysgenesis, and 100 controls. 4 missense mutations identified in POF patients, none in controls.	143
SALL4	POI	Sal-like protein 4	Transcription factor, maintenance and self-renewal of embryonic and hematopoietic stem cells.	20q13.13-q13.2	Wang, et al. 2009: Screened SALL4 coding regions for mutations in 100 Han Chinese women with non-syndromic ovarian failure and discovered two novel non-synonymous variants in the SALL4 gene: c.541G>A (p.Val181Met) and c.2449A>G. (p.Thr817Ala).	144
SETX	POI	Senataxin; AOA2, KIAA0625; ALS4, "amyotrophic lateral sclerosis 4", SCAR1, "spinocerebellar ataxia, recessive, non-Friedreich type 1"	Probable RNA/DNA helicase. Role in diverse aspects of RNA metabolism and genomic integrity. Essential for male meiosis.	9q34	Lynch, et al. 2007: Sequence analysis of FMR1, ATM and SETX genes in patient with ataxia with oculomotor apraxia type 2 and POI. Homozygous 6,292C→ T causing a premature truncation of SETX (R2098X). Homozygous for 1274G→ C in exon 10, causing G→ A at aa 425, unknown clinical significance. 6 other homozygous benign polymorphisms. Gazulla, et al. 2009: Two siblings with ataxia with oculomotor apraxia type 2 (AOA2), one with POI. Homozygous frameshift mutation in SETX, 2755_2756delGT, responsible for a premature stop codon at position 2760.	145, 146
SH3PXD2B	ANM	SH3 and PX domain-containing protein 2B	Involved in invadopodia and podosome formation, cellular motility, role in adipocyte formation.	5q35.1	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs11738223, effect -0.12 (0.02), p=2.0E-08	1
SHBG	POI	Sex hormone binding globulin	Binds and transports 5-alpha-dihydrotestosterone, testosterone, and 17-beta-estradiol.	17p13	Hogeveen, et al. 2002: Patient with hyperandrogenism during pregnancy, SNP P156L causing abnormal glycosylation and secretion of SHBG, found in 4 other patients with PCOS, idiopathic hirsutism or POI.	147
SLCO4A1	ANM	Solute carrier organic anion transporter family member 4A1	Sodium independent transport of organic anions, e.g. thyroid hormones, oestrone-3-sulphate.	20q13.33	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs2236553, effect -0.16 (0.03), p=4.4E-10; rs13040088, effect -0.16 (0.02), p=1.9E-10; rs140267842, effect=0.79 (0.14), p=1.60E-08	1

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SMAD7	ANM	SMAD family member 7, "MAD, mothers against decapentaplegic homolog 7 (Drosophila)", MADH7, MADH8, "SMAD, mothers against DPP homolog 7 (Drosophila)"	Inhibits TGF-beta signalling, which controls proliferation and differentiation of cells.	18q21.1	He, et al. 2010: Evaluation of 278 candidate genes in 12,723 women (Nurses' Health Study and Women's Genome Health Study studies). P-value adjusted for LD and number of tests. rs4939833, effect -0.4 years, p=0.029	12
SOHLH2	POI	Spermatogenesis-and oogenesis specific basic helix-loop-helix protein 2	Probable transcription factor, may be involved in spermatogenesis and oogenesis.	13q13.3	Qin, et al. 2014: Novel variants in SOHLH2 in women with POI of Chinese (n=364) and Serbian origin (n=197), with ethnically matched controls. 11 novel heterozygous variants including p.Glu79Lys (2 cases), p.Glu105Gly, and p.Thr321Pro, found in 4 Chinese POF cases; p.Leu120Phe (3 cases) and p.Leu204Phe, in 4 Serbian women.	148
SPPL3	ANM	Signal peptide peptidase-like 3	Aspartic protease, cleaves type II membrane signal peptides.	12q24.31	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs551087, effect -0.13 (0.02), p=3.9E-08	1
SRD5A1	ANM	3-oxo-5-alpha-steroid 4-dehydrogenase 1	Converts testosterone, progesterone and corticosterone into 5-alpha-3-oxosteroids	5p15.31	He, et al. 2010: Evaluation of 278 candidate genes in 12,723 women (Nurses' Health Study and Women's Genome Health Study studies). P-value adjusted for LD and number of tests. rs494958, effect 0.23 years, p=0.037	12
SRSF9	ANM	Serine/arginine-rich splicing factor 9	Constitutive splicing, selection of alternative splice sites.	12q24.31	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs551087, effect -0.13 (0.02), p=3.9E-08	1
STAG3	POI	Stromal antigen 3	Subunit of cohesin ring which ensures sister chromatid cohesion during meiosis	7q22.1	Caburet, et al. 2014: Large consanguineous Middle-East family with POF. Whole exome sequencing. Homozygous 1-bp deletion causing frameshift mutation in STAG3 and truncation of protein. Homozygous mutant mice were sterile.	149
STAR	ANM	Steroidogenic acute regulatory protein, mitochondrial	Steroid hormone synthesis, mediates the transfer of cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane where it is cleaved to pregnenolone.	8p12	Stolk, et al. 2012: HapMap imputed GWAS meta-analysis 38,968 women of European descent, with replication in up to 14,435 women. rs2517388, effect 0.262 years, p=9.31E−15. Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs2720044, effect -0.29 (0.03), p=7.3E-22	18, 1
STARD3	ANM	StAR-related lipid transfer protein 3	Binds and transports cholesterol.	17q12	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs2941505, effect -0.13 (0.02), p=1.9E-09	1

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
STX6	ANM	Syntaxin-6	Intracellular vesicle trafficking.	1q25.3	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry, rs1411478, effect -0.13 (0.02), p=1.4E-10	1
SYCE1	POI	Synaptonemal complex central element 1	Component of synaptonemal complexes formed between homologous chromosomes during meiosis	10q26.3	McGuire, et al. 2011: 89 POF patients. Microdeletion in 1 patient causing haploinsufficiency for SYCE1. de Vries, et al. 2014: Consanguineous Israeli-Arab family, genotyping in two sisters with POI (at 16 and 17 years), their parents and four siblings; 90 ethnically matched controls. Exome sequencing. Nonsense homozygous mutation (c.613C>T) in SYCE1, not in unaffected or controls.	32, 150
SYCP2L	ANM	Synaptonemal complex protein 2-like	Expressed in ovary.	6p24.2	He, et al. 2009: GWAS in 9,112 Europeans (Nurses' Health Study, Women's Genome Health Study studies). Rs2153157, effect 0.29, p=5.1E-08 Stolk, et al. 2012: HapMap imputed GWAS meta-analysis 38,968 women of European descent, with replication in up to 14,435 women. rs2153157, effect 0.165 years, p=7.76E−12. Perry, et al. 2014: Bi-variate analysis of menarche and menopause in 21,505 women, replication in 19,851 women. rs9467921, p=4.02E-11. Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs6899676, effect -0.21 (0.03), p=6.2E-16; rs9393800, effect -0.14 (0.02), p=1.1E-09	25, 18, 72, 1
TAC3	ANM	Tachykinin-3	Critical central regulator of gonad function.	12q13.3	Stolk, et al. 2012: HapMap imputed GWAS meta-analysis 38,968 women of European descent, with replication in up to 14,435 women. rs2277339, effect −0.380 years, p=2.47E-19. Chen, et al. 2014: Replication GWAS in 6510 African American women, rs2277339, effect 0.36 years, p=0.022 Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs2277339, effect -0.31 (0.03), p=1.8E-19	18, 11, 1
TDRD3	ANM	Tudor domain-containing protein 3	Scaffold protein in nucleus and cytoplasm. Recognises asymmetric dimethylation associated with transcriptional activation.	13q21.2	Stolk, et al. 2012: HapMap imputed GWAS meta-analysis 38,968 women of European descent, with replication in up to 14,435 women. rs4886238, effect 0.170 years, p=9.53E−11. Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry, rs4886238, effect -0.18 (0.02), p=2.5E-16	18, 1
Telomere length	ANM	n/a	Telomeres maintain the ends of chromosomes during replication.	n/a	Gray, et al. 2014: Leukocyte telomere length in 486 women aged 65+ years.10.2 months increase in ANM per 1 kb telomere increase (95% CI 1.3, 19.0).	151

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
TGFBR1	ANM	Transforming growth factor-beta receptor type-1	Part of receptor for the TGF-beta cytokines TGFB1, TGFB2 and TGFB3, involved in cell cycle, proliferation, differentiation	9q22	He, et al. 2010: Evaluation of 278 candidate genes in 12,723 women (Nurses' Health Study and Women's Genome Health Study studies). P-value adjusted for LD and number of tests. rs1590, effect 0.18 years, p=0.042	12
TGFBR2	POI	Transforming growth factor beta receptor II	Part of receptor for the TGF-beta cytokines TGFB1, TGFB2 and TGFB3, involved in cell cycle, proliferation, differentiation	3p22	Ma, et al. 2014: 1844 Chinese women, analysed polymorphisms in TGFRBR2 and miR-518. TGFBR2 rs3773661 associated with POF, OR=0.66 (95% CI 0.47,0.94) per minor allele C (p = 0.023).	152
TGFBR3	POI	Transforming growth factor, beta receptor III	Receptor which often functions as a co-receptor with other TGF-beta receptor superfamily members.	1p33-p32	Qin, et al. 2012: 112 Chinese women with POF, 110 controls, screened coding and flanking regions of TGFBR3 gene. 28 sequence variants, 12 novel. 3 missense, 2 synonymous, 7 intronic.	153
TLK1	ANM	Serine/threonine-protein kinase tousled-like 1	Involved in chromatin assembly processes.	2q31.1	Stolk, et al. 2012: HapMap imputed GWAS meta-analysis 38,968 women of European descent, with replication in up to 14,435. rs10183486, effect −0.196, p=2.21E−14 Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs930036, effect -0.19 (0.02), p=3.1E-19	18, 1
TNF	ANM	Tumour necrosis factor; DIF, "TNF superfamily, member 2", TNF-alpha, TNFSF2	Cytokine that binds the receptors TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR to induce cell death.	6p21.3	He, et al. 2010: Evaluation of 278 candidate genes in 12,723 women (Nurses' Health Study and Women's Genome Health Study studies). P-value adjusted for LD and number of tests. rs909253, effect 0.2 years, p=0.0034	12

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
U2AF2	ANM	Splicing factor U2AF 65 kDa subunit	Binds polypyrimidine tract of introns during spliceosome assembly.	19q13.42	He, et al. 2009: GWAS in 9,112 Europeans (Nurses' Health Study, Women's Genome Health Study studies). 6 SNPs at this locus. rs1172822 had smallest p, effect -0.49 years, p=1.8E-19. Candidates: BRSK1, HSPBP1, SUV420H2. Stolk, et al. 2009: GWAS in 2,979 Europeans (Rotterdam, Twins UK studies). 4 SNPs at this locus. rs1172822 had smallest p, effect=-0.4 years, p=6.28E-11. Candidates: BRSK1, TMEM224, SUV420H2. Stolk, et al. 2012: GWAS meta-analysis in 38,968 Europeans, replication in 14,435. rs11668344, effect -0.4 years, p=1.45E10-59. Candidate: TMEM150B. Other genes: BRSK1, HSPBP1, COX6B2, LOC284417, IL11, SUV420H2 Chen, et al. 2012: Replication GWAS in 3468 Hispanic women, rs17782355, effect -1.4 years, p=0.0064. Shen, et al. 2013: Replicated rs7246479 in GWAS of 3,533 Chinese women. Effect= 0.49 years, p=3.75E-05. Replicated rs1172822. Effect= -0.6 years, p=6.64E-05. Chen, et al. 2014: Replication GWAS in 6510 African American women, rs11668344, effect -0.31 years, p=6.54E-04 Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs11668344, effect -0.41 (0.02), p=4.2E-84; rs2547274, effect -0.22 (0.04), p=2.7E-08; rs12461110, effect -0.15 (0.02), p=5.0E-14	1; 25, 17, 26, 11
UBE2MP1	ANM	Homo sapiens ubiquitin-conjugating enzyme E2M pseudogene 1 (UBE2MP1), non-coding RNA.	Ubiquitin-conjugating (E2) enzyme pseudogene.	16p11.2	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs12599106, effect -0.12 (0.02) years, p=3.1E-08	1

Gene	Type	Other names	Function	Location	Reasons for being a ANM/POF candidate gene	Ref
UIMC1	ANM	BRCA1-A complex subunit RAP80	Binds 'Lys-63'-linked ubiquitinated histones H2A and H2AX at DNA lesion sites.	5q35.2	He, et al. 2009: GWAS in 9,112 Europeans (Nurses' Health Study, Women's Genome Health Study studies). 5 SNPs at this locus. rs365132 had smallest p, effect 0.4 years, p=8.4E-14. Candidates: UIMC1, UNC5A, HK3 Stolk, et al. 2012: GWAS meta-analysis in 38,968 Europeans, replication in 14,435. rs365132, effect 0.29 years, p=9.11E-32. UIMC1 best candidate. Stolk, et al. 2012: HapMap imputed GWAS meta-analysis 38,968 women of European descent, with replication in up to 14,435 women. rs365132, effect 0.287 years, p=9.11E−32. Shen, et al. 2013: Replicated rs365132 in GWAS of 3,533 Chinese women. Effect= 0.28 years, p=0.001. Chen, et al. 2014: Replication GWAS in 6510 African American women, rs402511, effect 0.21 years, p=0.042 Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs2241584, effect -0.14 (0.02), p=3.2E-11; rs365132, effect -0.24 (0.02), p=7.9E-33	25, 18, 27, 11, 1
VEGFA	POI	Vascular endothelial growth factor A	Growth factor, increases endothelial cell proliferation, role in angiogenesis and vasculogenesis.	6p12	Jeon, et al. 2011: Frequency of VEGFA polymorphisms (-2578C>A, -1154G>A, -634G>C, and 936C>T) in 135 Korean women with POF, vs 120 healthy controls. In POF, odds of genotype: -1154G>A OR=2.002 (95% CI 1.116,3.592; P = 0.019); combination genotype -2578CA+AA/-1154GA+AA OR=1.805 (95% CI 1.013–3.217; P = 0.044); haplotype -2578A/ -1154A haplotype OR=1.647 (95% CI 1.017,2.677; P = 0.041).	154
WNT4	POI	Wingless-Type MMTV Integration Site Family, Member 4	Involved in development. Ligand for members of the frizzled family of seven transmembrane receptors.	1p35	Chen, et al. 2011: Sequenced coding region and splice sites in 145 Han Chinese women with POF, 200 controls. Synonymous variation 195C>T with no change in aa sequence.	155
XPNPEP2	POI	X-prolylaminopeptidase 2 or APP2	Metalloprotease, role in inflammation and responses to injury and infection.	Xq26.1	Prueitt, et al. 2000: Balanced translocation disrupted an aminopeptidase gene, XPNPEP2.	156
ZNF729	ANM	Zinc finger protein 729	May play role in transcriptional regulation.	19p12	Day, et al. 2015: HapMap imputed GWAS meta-analysis, 69,360 women European ancestry , rs7259376, effect -0.11 (0.02), p=4.2E-08	1

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